Application Of Latent Variable Modeling And Related Techniques To The Analysis Of Toxicological Data

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By

Idunnuoluwa Okunola

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Head of the Department of the School of Public Health
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Abstract

Background: Soil contamination poses a significant problem in Canada because of either current or potential adverse impact on human health and the environment. Petroleum hydrocarbons (PHCs) are the most common sources of soil contamination in Canada and therefore the provision of remediation targets for such contaminated soils are of great concern to toxicologists.

Objective: This research project provides toxicologists with an alternative method for provisional remediation targets based on readily measured environmental variables without requirements for extensive toxicological testing. This study allows us to determine if models describing the relationships among soil characteristics, contaminant concentrations, and species responses could be used to predict these effects in soil when the contaminant concentrations and soil characteristics were known.

Methods: In this study, we used statistical methods to describe the relationship among soil characteristics, contaminant concentrations, and species responses, and how these can be used to predict toxicity in soils contaminated with petroleum hydrocarbons. Structural Equation Modeling (SEM) is a useful analysis tool that can be used to analyze these covariates, while accounting for those covariates that are intercorrelated, which are usually problems in current methods. Confirmatory factor analysis (CFA) under SEM was carried out using the lavaan package in R to estimate the measurement model which specifies the relationship between covariates and their latent factors and any inter-correlations between the covariates. A structural model was also analyzed to estimate the relationships between the latent factors. Non-linear procedures were carried out to quantify the relationships between PHC contaminant concentrations and the observed species responses to provide an estimate of the concentration at which there is a particular percentage change (IC_p; where p stands for the percentage change) in biological function for each endpoint (growth, reproduction, mass, shoot length, root length). Lognormal, exponential and gamma distributions were fit to the estimated IC_{25} and IC_{50} values using the "fitdist" function in the "fitdistplus" package of R software. A lognormal distribution gave the best fit to the IC_{25} and IC_{50} values.

Results: The CFA was carried out on different models specified based on theoretical

knowledge and the model with the best fit was identified. This CFA model specified that the masses (or other similar responses like size) of one species are indicators of the response of that species to the PHC contaminant in the soil and this response contributes to the aggregate response of all the other species to the PHC in the soil. Soil properties were added to this model to identify how some common soil properties affect toxicity. The amount of clay and the pH of the soil were found to be significant predictors of the aggregate response of the species. PHC concentrations were also found to be a significant cause of the aggregate response. IC_{25} and IC_{50} values were estimated for the two different study sites included in the dataset. The remediation guidelines for the PHC contaminated soils according to the IC_{25} values were estimated as 452.76 ± 50.38 *mg/kg* for site 1 and 234.93 ± 394.78 *mg/kg* for site 2. Therefore, PHC concentrations above these levels will be of great concern.

Conclusion: According to (CCME, 1996), the development of site-specific remediation objectives for PHC contaminated sites is a critical stage, and using current methods, requires extensive site-specific testing. This study demonstrated the utility of SEM in describing toxicity effects and most importantly the use of CFA in aggregating species endpoints to describe their joint response to PHC contamination. This method provides an alternative to current methods to estimate IC_{25} and IC_{50} values directly from the estimated aggregate species response. These values will then serve as remediation targets for toxicologists to use in risk assessments.

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List of Abbreviations

ANOVA	Analysis of Variance
CCME	Canadian Council of Ministers of the Environment
CWS	Canada-Wide Standards
EC_p	Effective Concentration for p% of individuals
IC_p	Inhibiting Concentration for a reduction in performance of p% compared to the control performance
LC_{25}	Lethal Concentration affecting p% of the individuals
LOEC	Lowest-Observed-Effect-Concentration
LOAEC	Lowest-Observed-Adverse-Effect Concentration
N/A	Not Available
NOEC	No-Observed-Effect-Concentration
NOAEC	No-Observed-Adverse-Effect Concentration
PHC	Petroleum Hydrocarbon Concentration
SEM	Structural Equation Modeling
SSD	Species Sensitivity Distribution
SSRO	Site Specific Remediation Objective

Chapter 1

Introduction

Across Canada, contaminated sites can pose a risk to human health, the environment, as well as other organisms in the environment. At the end of 2015, the Federal Contaminated Sites Inventory in Canada reported about 4,700 soil sites that were actively confirmed as contaminated by petroleum hydrocarbons (PHCs) (FCSI, 2015). There is a great concern for the effects of contaminants on the environment, which has led to the "development of risk assessment methodologies that require exposure and effect data inputs to characterize risk" (Hooper, 2008). In this study, we consider statistical methods to assess biological responses to soil characteristics and contaminant concentrations. The main objective is to evaluate the effects of these variables on soil toxicity. This would help us to understand the interrelationships between these variables, and to develop hypotheses based on statistical methods for prediction.

1.1 Background of the study

Present in this section are some key concepts related to soil contamination, the risks associated with soil contamination, and remedial recommendations by the Canadian Councils of Ministers of the Environment.

1.1.1 Soil Contamination and Associated Risks

Since the beginning of industrial development, various chemical compounds have been produced either by synthesis of chemicals or as by-products from the production of other commodities. The wastes from the production of these chemical compounds usually end up in the soil, water treatment plants, and rivers, where they accumulate and put the environment

at risk. A contaminated site according to the government of Canada (CCME, 2006) is one at which certain substances are present at concentrations above normal levels (specified in policies and regulations) and are likely to or already pose an immediate or long-term risk to human health or the environment.

Soil contamination poses a significant problem in Canada because of both its current or potential adverse impact on human health and the environment. Contamination inhibits natural processes that allow the soil to maintain its balance. Effects of soil contamination include, but are not limited to, loss of biodiversity and functions, declining soil fertility and changes in soil structure (Kukreja, 2016; Valentin *et al.*, 2013). Remediation of soils across the globe has proven to be time-consuming and expensive. A contaminated site should be considered as cleaned when the concentrations of the components making up the contaminant are at levels that are no longer toxic to soil biota and humans (Atlas, 2011). Several factors contribute to the expense of soil remediation, and these include, the type of soil, the quantity of contaminant released into the soil and the chemical/ physical properties of the contaminant (Stephen, 2012). The provision of remediation targets for contaminated lands is of great concern to toxicologists, and this can be more effectively done by determining the relationships between soil properties, and environmental covariates.

1.1.2 Petroleum Hydrocarbons and Soil Contamination

This study is focused on soils contaminated with PHC fractions. PHCs are the most common soil contaminants in Canada and the primary constituents of geological substances including crude and refined fuels and lubricants (CCME, 2008). Canada uses more than 1.7 million barrels of oil every day. Products varying from gasoline to heavy fuel oils (used in marine diesel engines) to asphalts (for constructing roads, railway beds, ports and building floorings) are refined from crude oils (Atlas *et al.*, 2011). These refined oils vary in viscosity, density, volatility and toxicity. PHCs are deposited into the environment during drilling, storage or transport, and during industrial activities (Edwards, 1983). PHCs consist of a broad range of organic compounds that are released into the environment in various proportions. According to Petrov (1987), 70 percent of the total mass of petroleum is hydrocarbons. The chemicals that make up hydrocarbons are classified as either aliphatic compounds, or

aromatic compounds (Table 1.1). Aliphatics have branched or straight-chained structure types and can either be saturated or unsaturated, whereas aromatics have one or more benzene rings in their structure. Aliphatic compounds have been found to pose a non-carcinogenic risk while aromatic compounds like benzene, ethylbenzene, and naphthalene can pose a carcinogenic risk.

Aliphatic hydrocarbons can be subdivided into three different groups based on their structure: alkanes, alkenes, and alkynes. Alkanes are also known as saturated hydrocarbons, and they have either straight or branched chain structures with single carbon-carbon bonds. Alkenes are known as unsaturated hydrocarbons, also having either straight or branched chain structures but possessing double carbon-carbon bond types. Alkynes like other aliphatics have straight or branched chains, but they possess triple carbon-carbon bond types. Alkanes are the most volatile of the three aliphatics, with alkynes being the least volatile. This property makes alkanes the first type of hydrocarbons that are quickly lost in contaminated soil. Aromatics are less volatile than aliphatics because they have higher boiling and melting points.

Because PHCs are complex mixtures of chemicals, risk assessments for these products are based specifically on toxic constituents, which are mostly the aromatic hydrocarbons. The Canada-Wide Standard for petroleum hydrocarbons in soil has sub-divided PHCs into four different fractions based on their number of carbon atoms, their physical and chemical properties, and their risk levels. These four fractions are described below (Korbas, 2013).

- Fraction 1 (F1) consists of aliphatics and aromatics with carbon chain length C_6 to C_{10}. F1 concentrations are found in water and are known for their high level of volatility and solubility.

- Fraction 2 (F2) consists of aliphatics and aromatics with carbon chain length $>C_{10}$ to C_{16}. F2 concentration levels can be found both in air and water but at lower concentrations in water compared to F1.

- Fraction 3 (F3) have carbon chain length $>C_{16}$ to C_{34}. F3 fractions are known to be scarcely soluble in water but not very volatile, and so F3 fractions are usually not regulated for water. F3 concentration levels are often found in soils.

Table 1.1: Classification of Hydrocarbons (Information assessed with permission from Fetter (1999)).

Hydrocarbon Class	Structure Type	Carbon Bond Type	Other names	Examples		
Aliphatics						
Alkanes	Straight or branched chain	Single	saturated hydrocarbons, paraffins, saturates	Methane, propane, hexane, ethane	$CH_3 - CH_3$	*Ethane*
Alkenes	Straight or branched chain	Double	unsaturated hydrocarbons, olefins	1-Butene, ethene	$CH_2 = CH_2$	*Ethene*
Alkynes	Straight or branched chain	Triple	Acetylenes	Ethyne	$HC \equiv CH$	*Ethyne*
Aromatics	Contains a Benzene ring	Any, but contains at least one benzene ring		Polycyclic Aromatic Hydrocarbons, polychlorinated biphenyls (PCBs)		*Benzene* C_6H_6

- Fraction 4 (F4) has carbon chain length $>C_{34}$ to C_{50}. F4 fractions are usually not volatile and are transported very minimally in the environment. Fraction 4 represents a significant proportion of petroleum products and crude oils. They are heavy and hardly soluble causing it to be difficult for them to be taken up by organisms.

Interactions between humans and the environment usually lead to various forms of environmental pollution, many of which may be hazardous. The long-term production and use of petroleum products have led to widespread contamination, making petroleum hydrocarbon contamination one of the most common environmental pollutions in the world (Del Panno *et al.*, 2005). According to CCME (2001), six out of every ten polluted sites are contaminated as a result of petroleum hydrocarbons and so petroleum hydrocarbons should be treated as priority pollutants. Humans and the environment are subject to health risks due to direct and indirect exposures to these contaminants. To understand the extent of the risks posed

by these contaminants, it is important to know the fate of these contaminants when they enter the environment. In the occurrence of soil contamination, crude and refined oils are subject to some physical, chemical and biological processes that affect their composition and their impact on the environment. Some of the commonly observed processes are described below.

- **Volatilization.**

  In this process, components of crude oil with lower densities are lost more quickly than components with heavier densities. As the lighter constituents evaporate, the portion of the oil that remains becomes heavier and thicker. One advantage of the evaporation process is that it reduces toxicity to some extent, while on the other hand, it increases the persistence of the residual crude oil in the soil. Higher temperatures are essential to speeding up the process of evaporation.

- **Adsorption.**

  Adsorption takes place in viscous substances like crude oil. This process occurs when molecules of crude oil bind to the particles of the soil. Adhesion of the crude oil molecules to the soil particles is strongest in organic soils and weakest in sandy soils. According to Mackay and Roberts (1985), adsorption can drastically reduce the mobility of contaminants in soils.

- **Photodegradation.**

  Some oil components like the polycyclic aromatic hydrocarbons (PAHs) react with sunlight. Photodegradation occurs when the soil is exposed directly to sunlight causing a breakdown of the hydrocarbon molecules. Components of crude oil that have found their way deeper in the soil profile are not affected by this process.

- **Dissolution.**

  There is a tendency that some of the acutely toxic components of the crude oil, which are the lighter hydrocarbons, released into the ground can dissolve into groundwater or surface water. When this happens, these components accumulate in fish and other

invertebrates, and they metabolize them making them toxic to other aquatic life and animals, including humans, who consume the fish.

- **Biodegradation.**

  Crude oil is a natural product and is a collective term used to classify oils generated from algae and plant material that long ago used the process of photosynthesis through sunlight as their source of energy. Over millions of years, the algae were heated at high pressure to produce oil containing the energy generated by the photosynthetic activity of the algae (Atlas, 2011). Because of this, oil products do not only serve as useful fuels; they also act as food for microbes (bacteria and fungi) in the soil. Oils contain high carbon concentrations, and so when they are released into the environment, energy microorganisms in the soil use the organic compounds in the oils as a carbon or food source to metabolize and biodegrade them (Atlas, 2011). Crude oil has variable degrees of biodegradability depending on how heavy the components are. Lighter crude oils are almost entirely biodegradable, but heavier crude oil components have very low levels of biodegradability. The extent of biodegradation of crude oils is also dependent not only on the properties of the components but also on the surface area of the spilled oil and the properties of the soil (pH, temperature, oxygen, pressure).

It is unsafe to assume that the effects of oil spills are largely taken care of in the soil by microbial activity or by the above mentioned physical and chemical processes. The process of degradation of oil spills by microbes is dependent on specific and sometimes changing environmental conditions to satisfy the needs of the microbial complexes, and so the degradation process may not be sufficiently fast to mitigate ecological damage. Also, the physical and chemical processes are not only dependent on the environmental conditions but also on the oil components. The dispersion can create room for increased exposure of biota to the toxic compounds in the oil (Atlas *et al.* 2011). Also, many oil dispersants have been found to be more toxic than undispersed oil (Shelton, 1971). Table 1.2 summarizes the different types of oils, their characteristics, and toxicity. Not all of the toxic components in oil disappear through volatilization; the lighter the oil, the more volatile it is. The heavier the oil, the longer it stays in the environment and the more easily it will be ingested by plants and

Table 1.2: Properties of Oil Types (Information assessed with permission from Klassen (2016)).

Density	Examples	Volatility	Toxicity	Clean-up procedures
Very Light oils	Jet Fuels, Gasoline	Highly volatile (evaporates within 1 or 2 days)	Contains high concentrations of soluble toxic compounds	Cleanup is difficult
Light oils	Diesel, No. 2 Fuel Oil, Light Crudes	Moderately volatile (two-thirds of the spill amount evaporates within a few days)	Contains moderate concentrations of soluble toxic compounds	Clean-up can be very effective
Medium oils	Most crude oils	One-third of the spill amount evaporates within one day	Can cause severe and long-term contamination of intertidal areas	Clean-up is most effective if done immediately
Heavy oils	Heavy crude oils, No. 6 Fuel Oil, Bunker C	Little or no evaporation or dissolution	Can cause heavy contamination of intertidal areas; can adversely affect animals due to possibility of them ingesting or being coated with oil; possible long-term contamination of sediments	Weathers very slowly and so clean-up is difficult under any condition

animals.

Toxicity is a good measure to determine whether a soil site is actually "cleaned up". Human senses are not very reliable in telling whether a site has been cleaned up properly or not because humans will only judge based on whether the oil can be detected by smell, tasted or even seen and this judgment may vary from person to person. The problems of PHC contamination include risk to human and environmental health, transportation of lighter PHC into groundwater or air allowing for greater exposure, the persistence of the branched chain hydrocarbons in the environment, potential for fire hazards occurring, and degradation of soil quality affecting soil biota (CCME 2008). The rate at which problems affect the environment, however, depends on the source of the PHC, the environmental conditions of the contaminated site and how long ago the PHC was released into the soil at the site (Han & Yang, 2011).

1.1.3 Recommended Remedies

In April 1998 and November 1998, the Canadian Council of Ministers of the Environment (CCME) held workshops that came up with three recommendations for approaching contaminated sites (CCME, 2006):

- A three-tiered risk assessment framework for contaminated lands (CCME, 2006).

- A consistent risk-based approach to evaluate and set priorities for remediation of contaminated sites.
- Equal protection of human health and the environment.

The principal purpose of establishing Canadian soil quality guidelines was to make available to humans and ecological receptors a functioning ecosystem that is capable of maintaining environmental and health standards with as little degradation of environmental quality as possible (CCME, 2006; CCME, 2007). Canada has adopted the three-tiered risk assessment framework for assessing and remediating contaminated sites.

The Tier I procedure involves Preliminary Quantitative Risk Assessment. This approach is carried out by screening out low-risk sites based on their exposure concentrations according to soil quality guidelines. The application of Tier I procedures is however limited to the fact that some sites may have unusual conditions including high natural background concentrations, complex compositions of contaminants, or unusual exposure scenarios (CCME, 2007). For these sites, the Tier II procedure is recommended by CCME. The Tier II method uses specific objectives and takes into account specific environmental properties (CCME, 1996). The process involves modification of Tier I criteria to accommodate the exposure scenarios of that particular site. The final step of Tier 2 (Alberta Environment and Parks, 2016) involves a combination of species-specific estimates to develop an estimated species sensitivity distribution (ESSD), which is then used to derive quantitative site-specific remediation objectives (SSROs). A species sensitivity distribution (SSD) shows the variation in sensitivity of different species endpoints to a particular contaminant. Because sites may vary immensely based on land use and exposure scenarios, the procedures involved in Tier II are not implemented under a set framework. This challenge is what regulators, assessors, and managers face when they need to derive Tier II SSRO (Zajdlik, 2013). This challenge faced in developing these Tier 2 SSROs comes from the fact that there are usually changes in species responses from site to site as a result of the variations in site-specific soil variables. Because of the variation in soil and environmental variables among sites, it is also difficult to successfully apply an SSRO derived explicitly from one site to another site. Solving this problem will reduce the expense of developing SSROs on a site-by-site basis as soil/environment variables are cheaper

and easier to measure than formal toxicological testing.

Finally, the third tier procedure performs detailed quantitative human health and ecological risk assessment for contaminated sites (CCME, 1996). The development of SSROs for PHC contaminated sites is a critical stage, and it requires extensive site-specific testing (Lamb *et al.*, 2012) (see chapter 2, section 2.1). According to Lamb *et al.* (2012), site owners face a dilemma either to remediate a site to Tier I standards or to undertake the expense of a Tier III assessment and develop an SSRO.

1.2 Objectives of the Study

In this study, we consider statistical methods to aggregate species endpoints, describe the relationship between soil characteristics, contaminant concentrations, and biological responses, and determine how these can be used to predict toxic effects in soils contaminated with petroleum hydrocarbons (PHCs). This work involves the application of latent variable modeling and related techniques to the analysis of soil toxicological data comprising multiple species and endpoints. In particular, this study demonstrates the utility of structural equation modeling (SEM) to assess the responses of species endpoints to PHC contaminants in soil. SEM is a useful tool that can be used to analyze covariates while accounting for those covariates that are inter-correlated and usually pose difficulties in current statistical methods (Stantec Consulting Ltd., 2013; also see Chapter 3 of this thesis).

One of the objectives of this study is to develop a model that will aggregate multiple endpoints from different species into a single latent variable. This variable is then used to estimate a single IC_{25} value for the aggregate response of the species. The IC_{25} value represents an estimate of the concentration that causes a 25 percent change in quantitative biological function such as growth, reproduction or weight of a particular species (Environment Canada, 2007b). The process of developing this latent variable involves identifying the endpoints that respond to contaminant concentrations differently compared to other endpoints. This also leads to a synergy of all the endpoints included in the model for analysis, and therefore ensures a reliable estimate of IC_{25}.

The process of generating a common IC_{25} value leads to the following scientific objectives

of this thesis.

- To develop site-specific remedial objectives for soils contaminated with petroleum hydrocarbons based on readily measured environmental variables and soil characteristics.
- To assess toxicological responses by modeling the relationships among contaminant concentrations and either species endpoints or soil characteristics.
- To summarize the aggregate response of species using a single-valued measurement IC_{25}.

To address the above objectives, we consider structural equation modeling. This new approach provides greater insights into the interrelationships among the variables than would be achieved using the traditional approach of SSD method.

1.3 Thesis Structure

Chapter 1 contains the background information that establishes a need for the research required for this thesis and outlines the study objectives. Chapter 2 is a review of both current and standard methods used to assess the toxicity of PHCs in the environment. This chapter also discusses different methods that have been explored to model toxicological responses and develop remediation objectives for contaminated lands. Chapter 3 provides an overview of the structural equation modeling approach that was used in this study including estimation techniques. Chapter 4 shows the analysis of data to assess toxicological responses using current methods and also using the latent variable modeling approach. Both methods were compared, and the flaws in the current methods were outlined. Finally, Chapter 5 briefly discusses how the study has provided answers to the research questions. The strengths and limitations are also outlined in this chapter.

Chapter 2

Literature Review

The research challenge is to describe the effects of abiotic factors that interact with soil contaminants and influence the toxicological responses of organisms and ultimately to estimate the risk associated with the contamination at the site. These measurements of toxicity usually take into account variations in environmental conditions and the differences among soil organisms. The effects of pollutants on the environment can be efficiently investigated by performing a series of toxicity tests. Standard toxicity testing procedures according to Environment Canada (2005) are categorized as either single-concentration tests or multi-concentration tests which are discussed in the first sections of this chapter. This chapter also describes different methods that have been explored to model toxicological responses and develop remediation objectives for different contaminated lands.

2.1 Standard toxicity testing methods

Single concentration tests are used to assess contaminated soils by comparing the response of organisms in the test sample to the response in the control sample. Multi-concentration tests are designed to estimate endpoints such as the NOEC (no-observed-effect concentration), LOEC (lowest observed effect concentration), EC_p (effective concentration) or IC_p (inhibiting concentration). These tests are carried out by using some fixed concentration levels and control (Weber, 1991; Environment Canada, 2005). The EC_p is the concentration that is estimated to cause a specified toxic effect to p percent of the test organisms while the IC_p represents an estimate of the concentration that causes a p percent change in quantitative biological function such as growth, reproduction or weight (Environment Canada, 2007b). Canada-Wide Standards (CWS) for petroleum hydrocarbons use ecotoxicological assessment

endpoints such as the IC_{25}s and the EC_{25}s for the development of soil quality guidelines. The no-observed-effect concentration (NOEC) is the highest concentration at which survival for example in the test sample is not significantly different from survival in the control sample while the lowest-observed-effect concentration (LOEC) is the lowest concentration at which the mean response in the test sample is not significantly distinct from that in the control sample (Crane & Newman, 2000).

2.1.1 Regression methods to determine EC_p and IC_p

Toxicity effects in both the single- and multi- concentration tests can either be quantal or quantitative. EC_p describes quantal effects, whether lethal or sub-lethal while the IC_p describes quantitative effects. In quantal tests, each organism is classified as either affected (failed) or not (passed) using direct counting. The objective of conducting pass/fail tests is to determine if survival in the single treatment is significantly different from the control survival (Weber,1991). The endpoints estimated in quantal tests are either the median lethal concentration (LC_{50}) for lethal effects or the median effective concentration (EC_{50}) for sublethal effects. Endpoints are estimated by regression methods and sometimes hypothesis testing methods. Quantitative tests measure effects that are highly variable like the size or mass of the organism.

Quantal tests are used to determine whether an organism shows a predefined effect or not; for example, whether the organism lives or dies, after being exposed to the concentration of the contaminant being tested. Because the response is binary, results follow a binomial distribution. The number of organisms for each exposure concentration and the duration of exposure should be the same. To estimate an EC_p or an IC_p, it is important to have test concentrations above or below the endpoint.

Methods for testing the statistical significance of contaminant effects in single- contamination quantal tests depend on the experimental design. Fisher's exact test can be used to compare test responses to control responses for samples taken from one site with or without field replicates (Environment Canada, 2005). Logistic regression can be used to assess results obtained from sites with field replicates. In quantitative single-concentration tests, responses from samples taken from a single site with field replicates can be analyzed using a t-test

while samples taken from different locations with field replicates are analyzed using ANOVA (Analysis of Variance). If the ANOVA results showed a significant difference between the location responses and the control, further analysis would be conducted to determine which of the location responses are different from the control or to compare each site to the other sites. The former is carried out using Williams' test for ordered data and Dunnett's test for unordered data while the latter is done using the Dunn-Sidak test) (Environment Canada, 2005).

According to Environment Canada (2005), a perfect choice of concentrations can only be made if the outcome of the test were known in advance, so the investigator is forced to use judgment. Better judgment can come from running a range-finding pre-test and performing the actual test by increasing the number of concentrations to be tested, allowing for a constant multiplier between each concentration - for example, 6, 12, 24, 48 and so on. The resulting concentrations used will be logarithmic in nature, and so the use of the logarithm of concentration is suitable for analysis of toxicological exposure and is usually the standard transformation in toxicity tests. Sometimes, probit transformation is used in addition to linearizing the relation with log concentration. One advantage of transformation is that it keeps the model as simple as possible, conserving the number of degrees of freedom, increasing the power of the model, and sometimes even widening the confidence limits (Anderson et.al., 1998).

Estimations for the EC_p are obtained either by maximum likelihood estimation (MLE) or by the method of iterative probit or logit regression. The latter involves using iteratively reweighted regression to arrive at an estimate. MLE is the better method between the two methods because it assumes that there is a proportion of organisms that will be affected at each level of concentration and the proportion of organisms affected is cumulative as the degree of concentration increases. The idea behind the MLE is that it attempts to produce estimates with the highest likelihood of observing the sample data. Both methods can be carried out using statistical software (e.g., SAS or SPSS). Other methods include the Spearman-Kärber, Binomial method, Moving Average method and Litchfield-Wilcoxon Graphic method (Environment Canada, 2005). One disadvantage of regression methods is that inappropriate transformations may distort concentration-effect relationships.

The estimated EC_ps or IC_ps are used in current methods to develop a species sensitivity distribution (SSD). This process uses nonlinear procedures to quantify the relationships between contaminant concentrations and the cumulative IC_{25} values of all the endpoints. Final steps involve computing a single IC_{25} based on the cumulative distribution of these individual IC_ps. SSDs are useful for many purposes, including quantifying the relationships between contaminant levels and species responses, and generating predictions about the proportion of species that will be affected if exposed to a particular concentration of the contaminant (Norton *et al.*, 2009). The SSD method is clear, easy to understand, requires simple statistical methods for analysis and is currently used for decision making by risk assessors (Angell *et al.*, 2012). The method however, has some disadvantages, which are not limited to the fact that there is no proof of its reliability compared to other methods, it requires relatively large datasets and some species may be overrepresented (Whitacre, 2010). Finally, IC_{25}s gotten from the SSD method to derive Canada-Wide Standards (CWS) for PHC do not consider confidence limits and according to Angell *et al.* (2012), this can result in false confidence. This present study focuses on an improvement to the SSD method.

2.1.2 Hypothesis testing methods to determine NOEC and LOEC

Hypothesis testing is usually carried out to determine reasonable estimates for the NOEC/ LOEC in multi-concentration tests. Hypothesis testing identifies statistically significant differences in responses between each test concentration and the control. The effectiveness of using a hypothesis test to detect statistically significant differences depends on whether there is a biological response and also on the design of experiment (for example, the number of replicates for each concentration levels, and the difference in the concentration levels). There are some reasons why the NOEC or LOEC are not excellent endpoints in toxicology (Chapman *et al.*, 1995, Crane & Newman, 2000). One reason is that there is a tendency that the higher the variability within the test or the fewer the replicates per concentration, the higher the NOEC. Another reason is that the NOEC is not necessarily the safest level of that concentration in the environment because hypothesis testing may not be able to detect a test concentration that produces a significant biological effect. Due to its mode of application, hypothesis tests cannot provide NOECs/LOECs outside the concentrations

that have been tested. The hypothesis testing method produces a pair of concentrations for the NOEC/LOEC endpoints instead of just one. The geometric mean of the pair is what is used in practice to provide a single endpoint. This single endpoint is referred to as the TOEC (threshold-observed-effect concentration). In the midst of these limitations, the advantage of the NOEC over regression-derived EC_p is that it is easier to calculate and easier to understand (Crane & Newman, 2000).

In recent toxicology research, hypothesis testing methods were used to determine the NOAEC (no observed adverse effect concentration) and the LOAEC (low observed adverse effect concentration) for PHC in a soil site for the purpose of soil remediation. One challenge in soil remediation is to determine how to derive SSROs for a particular site; another major challenge is to be able to apply the SSRO of that site to another site when the contaminant concentrations and pedological characteristics of the sites are known.

Stantec Consulting Ltd. (2013) conducted a study to develop a reasonable approach to derive SSROs. The data used were from a site that failed the Tier II pass/fail criteria (Alberta Environment and Parks, 2016). The toxicity tests were performed with four plant species and two invertebrate species from 8 site soil samples and three controls. The tests gave a pair of values for each endpoint, and so the geometric mean distributions of the two values were used as threshold concentrations for the both endpoints. The 25th and 50th percentiles were determined from the plot by rank species sensitivity of the threshold concentrations. The 25th percentile provided the remedial objective for agricultural/residential land uses that would protect 75% of the species while the 50th percentile provided the remedial purpose for commercial/industrial land uses that would protect 50% of the species (Stantec Consulting Ltd., 2013). The remedial objectives for the contaminated soils derived using a species sensitivity distribution were lower than current Tier 1 standards for F3 in soil. Therefore, this approach was not pursued further because of the degree of conservatism.

2.2 Regression analysis to determine toxicity endpoints

More recent studies have been developed to explore the toxicity of petroleum hydrocarbon in soils using different approaches (Stephenson *et al.*, 2000). Harvey (2011) provides an example of the application of regression to examine toxicity. Similar to the goal of this study, Harvey's objective was to help site managers and assessors to incorporate site-specific data into site management guidelines. The soil sample used was collected from Macquarie Island, a sub-Antarctic Island in the south of Australia in an 80.4 cm by 5.4 cm polyvinyl chloride core and stored at 4^{o}C" (Harvey, 2011). The soil contained 22.2% water content by weight after quantitative analysis, 4.46% organic matter, 4.16% of >2mm gravel content, 22.14% of 1.0 - 2.0mm coarse sand content, 66.35% of 0.25 - 1.0mm of medium sand content, 5.65% of 0.125 - 0.25mm of fine sand content, and 1.70% <0.063mm of coarse silt content.

The soil was exposed to ten different concentrations (between 0 and 50,000 mg kg^{-1}) of Special Antarctic Blend (SAB) diesel fuel for 21 days. The contaminated soil was assessed for nitrification activity, denitrification activity, total soil respiration and substrate induced respiration (respiration due to sucrose). Sensitivity to SAB contamination was greatest for potential nitrification activity. Regression analyses were used to determine the SAB concentration (EC_{20}) reducing microbial activity by 20% for nitrification activity, denitrification activity, total soil respiration and substrate induced respiration. Harvey (2011) determined the fits of various models - linear, logistic, Gompertz, hormesis, exponential, sigmoidal - using the corrected sum of squared error ($SSE_{corrected}$) values for each model. Corrected sum of squared error ($SSE_{corrected}$) values are derived by the application of a correction factor to the sum of squared error (SSE) values to account for the variance in the EC_{20} value.

$$SSE_{corrected} = SSE + \frac{1}{(\sigma_{EC_{20}})^2(E\hat{C}_{20} - EC_{20})^2} \tag{2.1}$$

where SSE is the sum of squared error, EC_{20} is determined by the model, $\sigma_{EC_{20}}$ is the variance of EC_{20}, and $E\hat{C}_{20}$ is the estimated EC_{20} value.

To compare the fit between two models, the Akaike Information Criterion (AIC) was used (Akaike, 2011). The AIC indicates the relative fit of models by adjusting the log likelihood statistic for the number of floating parameters (Burnham and Anderson, 2002). The model

with the best fit was the one that gave the smallest value for AIC. The AIC value is generated by most statistical software packages but it is denoted by:

$$AIC = -2L + 2k \tag{2.2}$$

where L is the log likelihood statistic and k is the number of free parameters in the model.

Based on AIC values, the linear and logistic models fit the data equally well but the EC_{20} for the linear model was 1.2 mg kg^{-1} of SAB and 660 mg kg^{-1} SAB for the logistic model. The logistic model was determined to be best model however, because its EC_{20} value was the closer one to the estimated EC_{20} value. The logistic model was (Harvey, 2011):

$$y = \frac{b}{\left[1 + \frac{p}{1-p}\right] \times \left(\frac{x}{EC_p}\right)^a} \tag{2.3}$$

where y is the observed response value, x is the PHC concentration over the time of exposure, b is the control response, p is the 20% microbial activity effect value, EC_p is the concentration of PHC at which microbial activity is affected by p (20%), and a is a fitting parameter.

Based on the logistic model, potential nitrification activity was the most sensitive indicator with an EC_{20} of 190 mg kg^{-1}, substrate induced respiration was sensitive to SAB contamination with an EC_{20} of 16 mg kg^{-1}, potential denitrification activity was sensitive to SAB contamination with an EC_{20} of 950 mg kg^{-1}, and total soil respiration was sensitive to SAB contamination with an EC_{20} of 220 mg kg^{-1}.

2.3 The use of Distance-Based linear models

A recent task in soil biology involved understanding the relationship between abiotic and biotic soil properties across different land-use types and from small to larger spatial scales (Birkhofer *et al.*, 2011; Fierer & Jackson, 2006; Fierer *et al.*, 2009; Decaëns, 2010; Wall *et al.*, 2008). Birkhofer *et al.*, (2012) studied the relationships between abiotic soil properties and soil organisms using distance-based linear models (DistLM). This approach was useful in determining if abiotic soil properties are related to soil biota after accounting for sampling location and land-use type. They hypothesized that soil properties explain significant proportions of variation in the abundance and diversity of soil biota after removing variation

explained by location and land-use type (pastures and mown fields, managed forests of beech and conifers, unmanaged beech forests, meadows). Therefore, the DistLM was based on the null hypothesis of no relationship between the soil properties and variation and diversity in soil organisms.

First, univariate procedures were applied to the data to test the relationship between three indicator groups: locations as a continuous variable, land-use type as binary-coded variables and seven soil properties as continuous variables. Standard procedure in DistLM was used to normalize variables, which were measured at different scales-sampling locations and land-use type. This analytical method provided the proportion of variation explained in the similarities between sites based on each indicator group and soil biota abundance. Secondly, DistLM were used to fit location and land-use type to soil biota abundance or diversity extracting variation that comes from large-scale differences. The same models were used to test for the rest of the change in soil biota abundance or diversity.

Birkhofer and his group found that abiotic soil conditions explained a substantial proportion of the variation in soil organism abundance independent of measures of soil contamination or location. For each predictor group, the highest amount of variation was explained by soil biota abundance and diversity, followed by land-use type and then sampling area. Regardless of sample location and land-use type, an increase in clay content was associated with increased species richness of earthworms. Their study indicated that after accounting for heterogeneity resulting from large-scale differences among sampling locations and land-use types, soil properties still explained significant proportions of variation in fungal and soil fauna abundance or diversity. This further affirms the challenge faced in deriving Tier 2 SSROs where the variation in species responses is most likely due to differences in soil properties. The approach used was found to be rather conservative and underestimated the amount of explained variation. The authors encouraged future studies to derive more general relationships between soil properties and soil biota involving larger spatial scales and different land-use types.

2.4 Nuclear Magnetic Resonance (1H NMR) - based metabolomics approach

Petroleum hydrocarbons are one of the most common soil contaminants. PHCs are complex chemicals because they are made up of mixtures of different compounds, making it difficult to predict accurately the toxicity associated with PHC contamination using chemical extraction methods alone (CCME, 2008; Dandie *et al.*, 2010). Studies have shown that methods like the 1H NMR (Nuclear magnetic resonance)-based metabolomics have proven to be very useful especially in ecotoxicological studies (Robertson, 2006; Lehman-McKeeman, 2004; Brown et al., 2008; Whitfield-Aslund et al., 2013).

Metabolomics are used in ecotoxicological studies to provide information regarding the response of earthworms to sub-lethal doses of environmental contaminants (Brown, 2008). Whitfield-Aslund *et al.* (2013) carried out this method to examine earthworm responses to petroleum hydrocarbon exposure in an aged field-contaminated soil. They conducted their study using 1H NMR -based metabolomics and traditional ecotoxicity endpoints. The conventional ecotoxicity tests (survival, reproduction, and growth) showed that the soils were not acutely toxic to earthworms (average survival $\leqslant 90\%$). H NMR -based metabolomics revealed statistically significant relationships between earthworm metabolic profiles (collected after 2 or 14 days of exposure) and soil properties. Results showed a significant association between the earthworm metabolomic data and the reproduction endpoints (measured after 63 days). The authors concluded that metabolomic responses measured after short exposure periods may be predictive of toxicity endpoints for earthworms exposed to soil contaminants (Whitfield-Aslund et al., 2013).

2.5 The use of Probit Analysis

The present analytical framework for the development of Tier 2 SSROs involves the derivation of species and endpoint specific toxicity values such as Effect concentrations (EC_{50}s) and Inhibitory concentrations (EC_{50}s). These levels are important in determining the threshold effect concentration, which could serve as the SSRO for a site. Velicogna *et al.* (2012) assessed

the toxicity of a D5 (Decamethylcyclopentasiloxane) contaminated biosolid in an agricultural soil. Plant testing was done by evaluating the effects of D5 on seedling emergence, shoot, and root length, and shoot and root dry mass while invertebrate testing was carried out by assessing the impact of D5 on adult lethality, juvenile production, and individual juvenile dry mass (earthworms only). The plants that were tested were *Trifolium pratense* (red clover) and *Hordeum vulgare* (barley) while the invertebrates that were tested were *Eisenia andrei* (earthworm) and *Folsomia candida* (springtail). Effective concentrations (EC_{50}s) were calculated using the EPA (Environmental Protection Agency) Probit Analysis program. Inhibitory concentrations (IC_{50}s) were estimated using nonlinear regression analysis and in cases where model assumptions were not met, the Inhibition Concentration (IC_p) Approach (ICPIN) (version 2.0) was used. The toxicity of the D5 varied among the species and the endpoints. No significant effects were observed for *Trifolium pratense* or *Eisenia andrei* test endpoints; however, there were significant toxicity effects observed for *Hordeum vulgare* plant growth and *Folsomia candida* survival and reproduction (Velicogna *et al.*, 2012). All toxicity data were analyzed according to Environment Canada (2005) recommendations. The emergence of all plant species was not affected by D5 concentrations. There was no effect observed for any of the test endpoints for *Trifolium pratense* while for *Hordeum vulgare*, shoot and root biomass endpoints were significantly more affected than length. Chemical losses were observed at high concentrations, but this may have been due to differences in soil characteristics.

2.6 The Data Reduction and Model Averaging (DRAMA) approach

Developing Tier 2 SSROs can be very challenging, particularly due to interactions between the physical and chemical properties and the biological responses between and within site soils. Zajdlik, B. (2013) used toxicity tests from a plant (*Elymus lanceolatus*), an earthworm (*Eisenia andrei*) and a springtail (*Folsomia candida*) conducted across three studies contaminated with PHCs (petroleum hydrocarbon fractions). The DRAMA (Data Reduction and Model Averaging) approach was used to address the interaction and potential redun-

dancy between site physical and chemical properties by creating synthetic variables. Zajdlik explored the correlations between site physical and chemical characteristics, synthetic variables, contaminants and toxicity tests responses. Each toxicity test was conducted following Environment Canada methods (Environment Canada, 2004; 2007a). The soil variables measured are similar to the ones to be used in this study.

Zajdlik looked at the possibility that the toxicity of PHCs varied among the three studies. He modeled toxicity test responses using generalized linear mixed effects. Rather than search for a single best fitting model using one or more statistical criterion, model averaging (Claeskens and Hjort, 2008; Burnham and Anderson, 2002) was used to ensure that a particular model was not given undue superiority over other models and that the true model uncertainty was acknowledged. Zajdlik's study suggested that non-contaminant variables be considered in the estimation of Tier II SSROs. Data Reduction was carried out using non-PHC variables excluding texture, organic carbon, and organic matter categorical variables. There were significant interactions between the variables and at least one biological response for every variable and there were frequent significant correlations between total PHCs and at least one toxicity test for each species.

2.7 The Partial Least Squares (PLS) approach

The Partial Least Squares (PLS) approach is another well-used method, and can be used to describe the relationship between soil and contaminant variables and toxicity response. The utility of the Partial Least Squares (PLS) approach to predict individual toxicological endpoints from soil variables (including physical properties and contaminant concentrations) was investigated by Whitfield-Aslund (2012). PLS regressions were constructed with the NIPALS PLS algorithm using a matrix of measured soil characteristics as the 'X' multivariate predictor matrix and each of the ecotoxicity test endpoints as the 'Y' (response) matrix.

The PLS approach is an alternative to SEM in that it uses a principal components measurement model instead of the factor analytic measurement model as in SEM. In the principal components measurement model, the latent variables and their indicators are linearly related. The PLS method is more concerned with maximizing the predictive ability of the

model rather than the model fit. The PLS approach in this study was carried out using the leave-out-class-out cross-validation procedures to derive models. The number of components that explained the most variation of Y (R^2Y) was the number of elements that were in each final PLS model. The total explained variation of X (R^2X) and Y (R^2Y) was recorded for each PLS model to determine model fit. The R^2Y was reported as the predictive ability of the model. Significance testing for each model was estimated through response permutation testing (Eriksson *et al.*, 2006). After the authors evaluated model fit and the predicting power of the model, they affirmed that it was possible to link different soil properties to toxicity endpoints using the PLS approach. However, the authors highlighted that the predictive power of the models developed using this approach is most likely to be inadequate for soils with substantially different soil properties from the soils used in this study.

2.8 The Structural Equation Modeling (SEM) approach

Lamb *et al.* (2012) demonstrated the utility of confirmatory factor analysis to bring together different endpoints into a single latent variable that can be incorporated into standard procedures to estimate IC_p values. They also examined the potential for using SEM to develop models that can predict toxicological effects across sites, and can also account for the influence of common environmental factors. Little variation was explained by the cross-site models that were developed, and so the models were not used for predictive purposes. One impediment was sample size compared to the number of environmental factors. They recommended changes in sampling designs and an assembly of larger databases (20+ sites) containing covariates to ensure a sufficient and valid scope of inference.

2.9 Conclusion

The toxicity of contaminants like petroleum hydrocarbons has been accessed using different methods with the common goal of developing remediation guidelines for contaminated soils. Current recommendations by the Canadian Council of Ministers of the Environment (CCME) for cleaning up contaminated soils involve the adoption of soil quality guidelines. Due to

variation in soil properties from one soil site to another, a remediation objective implied by soil quality guidelines might not apply to all soil sites. The challenge in deriving site-specific remediation objectives (SSROs) is the basis for conducting this study. The first steps forward involve understanding the interactions among the contaminant concentrations in the soil, soil biota and environmental variables. Only then can the toxic effects of the contaminant be understood and remediation undertaken. Various methods have been used to better understand interactions among the contaminant concentrations in the soil, soil biota and environmental variables.

Standard toxicity testing use endpoints such as the NOEC, LOEC, EC_p or IC_p, which are used to develop soil quality guidelines. These endpoints measure concentrations that cause toxic effects in soil species, and they are usually estimated by regression methods. The EC_ps or IC_ps are most commonly used to develop what is called a species sensitivity distribution, which gives a clear picture of how sensitive the species are to the contaminant. Apart from regression analyses, various approaches have been explored to quantify the effects of contaminants in soils as described in this chapter; there are limitations inherent to all.

One of the aims of the research conducted herein is to provide a reasonable approach using latent variable analysis to assess responses of soil biota to petroleum hydrocarbon (PHC) contamination.

Chapter 3

Statistical Methods

The methodology used in this study is the Structural Equation Modeling approach (SEM). SEM is a potential solution for many of the problems encountered in the analysis of site-specific toxicological data (Lamb *et al.*, 2011) and has become commonly used in the natural sciences (Grace, 2006; Lamb *et al.*, 2012). Definitions of some terms and concepts that were employed in this study are given below:

- Latent Variable: A latent variable is a variable that is not directly observed or measured but can be deduced from other variables that are directly measured.
- Composite Variable: A composite variable represents the collective causal influences from multiple variables.
- Exogenous Variable: An exogenous variable is a variable that is not caused by another variable but rather is the cause of other variables.
- Endogenous Variable: An endogenous variable is a variable that is caused by other variable(s) in the model. (Kenny, 2011)
- Manifest variable: Manifest variables are also known as observed variables.
- Indicator: An indicator is an observed or manifest variable that is linked to either a latent or a composite variable (Grace & Bollen, 2006)
- Factor loadings: Factor loadings are estimates of direct effects. They are interpreted as regression coefficients.

3.1 Overview of the Structural Equation Modeling (SEM) approach

The structural equation model (SEM) is different from other statistical models because other statistical methods only provide estimates of associations while SEM attempts to represent scientific, cause-effect relationships. SEM is a modeling framework for studying and evaluating causal hypotheses and is different from the methods commonly used by toxicologists. It is a useful multivariate tool for assessing the reliability and validity of a model ("Advantages of SEM over regression", 2016). To investigate causal relationships in SEM, sufficient evidence is required to support the relationships between the variables in the model. SEM is based on the analysis of variances and covariances rather than raw data. The use of variance and covariances allows for the estimation of both standardized and unstandardized parameters (Grace, 2006).

One important aspect used in SEM is the concept of the latent variable. As defined in the previous section, a latent variable is a theorized variable that is not directly measured but is inferred from the observed variables that we assume to be correlated one way or the other with the latent variable. A latent variable can be assumed to behave as a variable with no observed values. In this study, latent variables were utilized to aggregate multiple endpoints from species and to evaluate whether all the endpoints used in the model were providing the same information. The primary latent variable employed in this study is the aggregate response variable describing toxicity, which represented the species responses to the concentrations of PHC in the soil. One advantage of using latent variables is that it allows to estimate and correct for measurement error.

Like in regular regression methods, not all relationships in SEM are considered linear. Non-linear relationships are usually put through the process of transformation before they can be incorporated into a model. However, in SEM, it is hard to linearize polynomial relationships. To be able to incorporate polynomial relationships in a structural equation model, we use a variable called a composite variable. Composite variables have the ability to model relationships that are non-linear (Grace & Bollen, 2008). These kinds of relationships

are not uncommon in toxicology, and so composite variables can be very useful.

The structural equation model consists of a measurement model and a structural (latent) model. The measurement model, also known as confirmatory factor analysis (CFA) describes the relationships between the latent and the observed variables while the structural model specifies the relationships among the latent variables. The primary objective of CFA is to provide an explicit framework to test the reliability of the relationship of the observed variables to the latent variable(s). Because SEM is theory-driven, analyses in this study are carried out based on theoretical knowledge of the relationships between observed and latent variables. SEM involves some steps, which were employed in this study and were followed in a logical order. Figure 3.1 gives the overall summary of the SEM methodology, the steps of which is discussed in subsequent sections of this chapter.

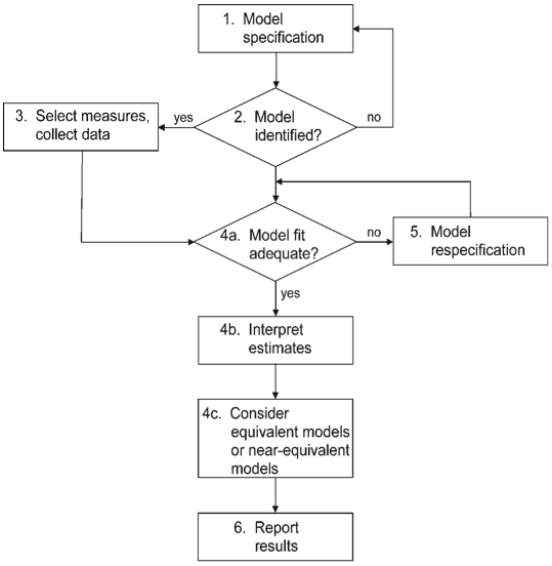


Figure 3.1: Flowchart for the basic steps of SEM: (Assessed with permission from Kline (2015)).

3.2 Model Specification

Model specification, the most important step in the SEM approach involves the representation of causal hypotheses in the form of a structural equation model. It involves using a set of paths to represent causal relationships in both the measurement model and the structural model. Model specification in this study was based on prior empirical knowledge of the species to be used in the analysis (both plants and invertebrates) and the soil system, and this is what distinguishes SEM from other linear modeling approaches. The models in this study were specified using two different methods - a visual method of drawing diagrams and a mathematical method of constructing series of equations. These diagrams, also known as causal/path diagrams provide a visual representation of possible causal relationships between the variables to be used.

The diagram used to specify the model makes use of symbols from the McArdle-McDonald reticular action model (RAM) symbolism (Kline, 2015; Markus, 2012) making the translation of the model even easier (Table 3.1). Rectangles in path diagrams are used to represent observed exogenous or endogenous variables, circles are used to represent latent endogenous or exogenous variables, and single-headed arrows are used to indicate direct effects of one variable to another variable. Double-headed arrows are used in two various ways; a double-headed arrow pointing from one variable to another variable signifies the covariance between the two variables while a smaller double-headed arrow pointing out of an exogenous variable and pointing back to the same exogenous variable indicates the variance of that variable.

The equations define the model's parameters, which correspond to presumed relations among the observed or latent variables; the parameters are eventually estimated using the sample data. The equations are probabilistic, and they are made up of the linkage form of the effect of the relationship between the species endpoints and the responses and the form of the responses. Linkage forms are usually either simple and linear or polynomial and curvilinear with linear terms.

Assumptions and Requirements in Structural Equation Models Statistical inference is carried out under the assumptions described below:

- Sample size: 'Rule of thumb': $N \geqslant 8K$ where K is the number of observed variables in

Table 3.1: Path diagram symbols.

Symbols	Name	Use
	Squares/rectangles	Represent observed variables
	Circles or ellipses	Represent latent variables
	Unidirectional arrowheads	Represent direct effect of one variable on another
	Two-directional arrowheads between two variables	Represent the covariance between two variables
	Two-way directional arrowheads on the same variable	Represent the variance of an exogenous variable

the model (Sigal, 2012). This requirement is quite restrictive because large datasets in toxicology rarely exist. Ecologists have discussed this issue extensively and advise that it is better to analyze with smaller datasets than not at all (Grace, 2006).

- Distribution Assumptions: Each dependent and mediating variable is assumed to be continuous and normally distributed; each observed variable (species endpoints) should be univariate normal; multivariate normality of endogenous variables is assumed. It is also assumed that the associations between variables are linear.

- The measurement error vectors are considered to be independent.

- The model must be identified

In summary, the ML estimation technique used in this study assumes multivariate normality of the continuous endogenous variables, the independence of the exogenous variables, residuals and scores, and the correct specification of the model.

3.3 Model Identification

A model is identified if a unique estimate can be derived for every model parameter (Kline, 2015). Identification is a property of the model and not the data and so properties of the data like sample size will not affect identification of the model. The two necessary requirements that were put in place to ensure our models were identified are: the degree of freedom of the models were at least zero, and one of the paths leading out of each of the latent variables were assigned a scale of 1.0. An identified structural equation model is identified and has the same number of free parameters as observations ($df_M = 0$) while an over-identified structural equation model is identified and has fewer free parameters than observations ($df_M > 0$). To test model fit, the model must be over-identified. Under-identification (non-identification) of a model occurs when it is not possible to uniquely estimate all of its parameters (Kenny, 2004), in this case, an element in the variance-covariance matrix.

3.4 Operationalize constructs: collect, prepare and screen the data

The data, after collection were tested for collinearity among the species and invertebrate endpoints, univariate and multivariate outliers, and also missing data. Operationalization involves defining variables into measurable factors. More contemporary forms of operationalism allow for both multiple indicators and disturbance terms for composites (Kline, 2015).

3.5 Model Estimation

The maximum likelihood estimation (MLE) technique is the most commonly used estimation method in SEM. Other methods include the partial least squares path modeling (SEM-PLS), the weighted least squares approach (WLS), and the generalized maximum entropy (SEM-GME) (Kline, 2015; Finch & French, 2015; Crisci, 2012). In this study approach, the structural equation model was analyzed using the maximum likelihood estimation method. MLE method is a normal theory method because multivariate normality is assumed for the

population distributions of the continuous endogenous variables. The MLE method is both scale-free and scale invariant only if the covariance matrix is analyzed, which is the case in this study. ML estimators are known as the best estimators (Finch & French, 2015; Grace, 2006). ML methods are consistent with solving parameter estimation problems in a large variety of situations, their estimators have unbiased minimum variance as sample size increases. The ML method estimates all parameters that minimize the following equation:

$$F_{ML} = \frac{1}{2} tr\left[\left(\left[S - \Sigma\right]\Sigma^{-1}\right)^2\right] \tag{3.1}$$

where S is the data-derived covariance matrix of the observed indicators and,
Σ is the covariance matrix of the observed indicators as predicted by the specified model.

The assumptions of ML estimators include:

- Standard ML estimation assumes unstandardized variables, and so the standard errors that are calculated from the model are for unstandardized solutions only.
- Independence of scores
- Model must be correctly specified. The more serious the specification error, the more serious the resulting bias.
- Data were assumed to follow a multinormal distribution
- The covariance matrices S and Σ are assumed to be positive definite.

The following procedures took place to determine the best estimates of the parameters:

1. Evaluation of model fit (if poor, we skip to step 5). This process involved determining how well the model explained the data. Global model fit in SEM is determined from the evaluation of the difference between the model-implied covariance matrix and the observed covariance matrix. The most fundamental test statistic that was used in this study is the model chi-Square (χ^2_M) statistic also known as the likelihood ratio chi-square. The χ^2 test helped to detect paths that should be included in the model. Non-significant paths are either included or excluded depending on the researcher. Excluding a non-significant path that was previously included in the model by theoretical

justification only means that the path is not important in this particular study (Grace, 2006). Care was taken while using the χ^2 test because it is affected by multivariate normality and sensitive to sample size (tends to increase even with a slight increase in sample size). According to Grace (2006), the chi-square is recommended when the sample size is less than 200. The model chi-square (χ^2_M) is the product of the fit function (the criterion minimized in ML estimation) and one less the sample size (Kline, 2015), which can be written mathematically as:

$$\chi^2_M = (N-1)F_{ML} \tag{3.2}$$

Approximate fit indexes that were used are Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI) and Standardized Root Mean Square Residual (SRMR). Each describes model fit from a different perspective. The RMSEA is scaled as a badness-of-fit index where a value of zero indicates the best fit; a value of at most 0.05 is desired. The RMSEA is an absolute measure of fit that follows a noncentral chi-square distribution. The noncentrality parameter allows for differences between the model-implied covariance matrix and the data-based covariance matrix up to the expected value of model chi-Square (χ^2_M) or model degree of freedom (df_M). When $\chi^2_M \leq \mathrm{df}_M$, the RMSEA = 0. The value of the RMSEA decreases and the width of its confidence interval decreases as sample size increases and model degrees of freedom increase. Its computational formula is:

$$RMSEA = \sqrt{\frac{\chi^2_M - df_M}{df_M(N-1)}} \tag{3.3}$$

The CFI is an incremental fit index. It estimates how much better the estimated model fits compared with a baseline model (B). When $(\chi^2_M) \leq (\mathrm{df}_M)$, CFI = 1.0. The CFI range between 0 and one where 1 indicates the best fit, and we want a value of at least 0.95. Hu and Bentler (1999) suggested using the CFI together with SRMR; both are based on the differences between the observed and predicted covariances.The formula for CFI is:

$$CFI = 1 - \frac{\chi^2_M - df_M}{\chi^2_B - df_B} \tag{3.4}$$

The TLI is another incremental fit index. The TLI uses the ratio of the chi-square value (χ^2) to its degree of freedom (df). The lower this ratio, the better the model fit; if the ratio of the χ^2 to the df does not change, the TLI remains the same. The TLI and CFI are highly correlated and are both dependent on the size of correlations in the data. The higher the correlations, the higher the TLI and CFI. A model with TLI of at least 0.95 is most desired. The TLI can be computed as:

$$TLI = \frac{\chi^2/df(nullmodel) - \chi^2/df(proposedmodel)}{\chi^2/df(nullmodel) - 1} \tag{3.5}$$

SRMR is an absolute measure of fit and is derived from the differences between observed and predicted covariances. An SRMR of 0 indicates perfect fit and a value less than or equal to 0.08 is most desired. The formula for SRMR is:

$$SRMR = \sqrt{\sum_j \sum_k r_{jk}^2 / p*} \tag{3.6}$$

where r_{jk} is the standardized residual from covariance matrix with j rows and k columns; $p*$ is the number of nonduplicated elements in the covariance matrix.

To compare models, we used the Akaike Information Criterion (AIC), which is a comparative measure of relative quality. The model with the lower values of AIC is usually considered as the better fitting model. For a model's AIC to be significantly different from the other model's AIC, there must be a difference of 2.0 (Bozdogan, 1987). The formula for the AIC is:

$$AIC = 2L + 2m = n\left[(\ln(2\pi)) + \ln\left(\frac{SSE}{(n-m)+1}\right)\right] + 2(m+1) \tag{3.7}$$

Where L is the log likelihood statistic; m is the number of floating parameters in the model; n is the number of observations; and

$$SSE = \Sigma(\hat{y} - y)^2 \tag{3.8}$$

2. Assuming satisfactory model fit, parameter estimates of effects were interpreted. Path coefficients were interpreted the same way that multiple regression coefficients are and were checked for significance using the z-test. The ratio of the observed variance in

the endogenous variable to the unstandardized variance of its disturbance was interpreted as the proportion of the total variability in the endogenous variable that is unexplained. Accordingly, one minus that fraction was reported as the proportion of the explained variance, which is equivalent to the squared multiple correlation (R^2_{smc}) for the endogenous variable.

3. We also considered equivalent or near-equivalent models that explain the data just as well as the preferred model but with a different configuration of hypothesized relations among the same variables.

3.6 Model Re-specification

Re-specification was guided more by rational considerations rather than purely statistical ones. Models were respecified in the case of inadequate fit by referring to a list of theoretically justifiable changes to the initial model. Each re-specified model was modified based on other presumed relationships or patterns among the observed and latent variables that can also be backed up by adequate knowledge of the soil system (Kline, 2015). Modification indices in SEM were also used to suggest new paths that could be included in the model to improve model fit.

Chapter 4

Data Analysis: Developing a Structural Equation Model (SEM) describing toxicity

4.1 Data and Variables

Toxicity tests were performed based on Environment Canada (2004) guidelines and carried out in a laboratory in an Ontario-based firm - Stantec Consulting Ltd., Guelph, Ontario. Petroleum Alliance Technology of Canada (PTAC) and its affiliate organizations collected data between 2000 and 2015. A total of 50 soil samples collected from 2 different study sites were used in this project (see Appendix A for full dataset). The test species were Northern Wheatgrass (*Elymus lanceolatus*), Barley (*Hordeum vulgare*) and Alfalfa (*Medicago sativa*) for plants; earthworm (*Eisenia andrei*) and soil arthropod (*Folsomia candida*) for invertebrates. Tests involved the measurement of plant and invertebrate endpoints over a range of the PHC concentration levels. Plant properties measured were seedling emergence, root length, shoot length, root dry mass, and shoot dry mass while invertebrate endpoints measured were survival, mass, and number of offspring. Soil properties measured across the soil sites were soil moisture, pH, conductivity, water-holding capacity (WHC), total nitrogen, total carbon, inorganic carbon, organic carbon, phosphorus, organic matter content, gravel, sand, very fine sand, fine sand, medium sand, coarse sand, very coarse sand, silt content and clay content.

The first site had a sample size of 34 while the second had a sample size of 16. The samples covered a range of concentrations of petroleum hydrocarbons (PHCs) and were taken from different locations at each study site. Toxicity testing in site 1 was conducted by exposing plants and soil invertebrates to the worst-case contaminated soil to identify if

adverse effects occurred while taking into consideration site-specific physical and chemical soil characteristics. The objective was to determine whether the soil samples satisfied the Tier II pass/fail criteria to decide which samples were of greatest concern and in need of remediation. Site 1 consisted of two phases: Phase 1 was an ecotoxicity assessment of PHC-contaminated site soils collected from a site in Alberta using one earthworm and *Folsomi Candida*, and three plant species (Barley, Northern Wheatgrass, and Alfalfa); and, Phase 2 was an ecotoxicity assessment with the soil in the phase 1 testing as the control, as well as soil samples that were contaminated with petroleum hydrocarbons, and metals like boron, copper, lead, and zinc. Site 2 also consisted of two phases: Phase 1 was an ecotoxicity assessment of PHC-contaminated site soils collected from a site in Alberta using one earthworm, one Collembola and three plant species (Barley, Northern Wheatgrass, and Alfalfa); and, Phase 2 was an ecotoxicity assessment with one earthworm, one collembolan, and three plant species (*Red Clover*, Northern Wheatgrass, and perennial ryegrass). The soils included both site and clean reference control soils, PHC-contaminated surface soils, and soils from the transition zone between contaminated and non-contaminated areas. The clean reference surface soils were labeled A and the clean reference soil from the transition zone was B. The contaminated surface soils were labeled C. The contaminated soils from the transition zones were labeled D. Three control site soils had levels of petroleum hydrocarbons and levels of all metals below Alberta Tier 1 Soil Remediation Guidance values (Alberta Environment and Parks, 2016) for all land-use classes. The reference soils and all site soils were classified as either fine-grained soils or coarse-grained soil.

Contaminant concentrations, soil properties and endpoints for earthworm, *Folsomi candida*, Barley, Northern Wheatgrass, and Alfalfa were complete for all the study sites and were used for analysis in this study (see Table 4.1 & 4.2 for full description). The relationship between these species endpoints measured, soil properties, and the PHC contaminants was explored. The objective of this study was to construct a model that links an aggregate species response variable based on the above endpoints to toxicant concentrations and also to measures of soil quality.

Table 4.1: Desciption of variables - endpoint variables.

Notation	Variable Name	Description
y_2	EA progeny No.	Number of fertile progeny produced by Eisenia andrei
y_3	EA progeny wet mass	The weight of Eisenia andrei progeny before drying
y_4	EA progeny dry mass	The weight of Eisenia andrei progeny after drying at 60 - 70 degrees C
y_5	FC progeny No.	Number of fertile progeny produced by Folsomia candida
y_6	FC survival	% of Folsomia candida that survived after tests were done
y_7	Ba root length	The root length of Barley
y_8	Ba root dry mass	The weight of Barley root after drying at 60 - 70 degrees C
y_9	Ba shoot length	The shoot length of Barley
y_{10}	Ba shoot dry mass	The weight of Barley shoot after drying at 60 - 70 degrees C
y_{11}	NWG root length	The root length of Northern Wheatgrass
y_{12}	NWG root dry mass	The weight of Northern Wheatgrass root after drying at 60 - 70 degrees C
y_{13}	NWG shoot length	The shoot length of Northern Wheatgrass
y_{14}	NWG shoot dry mass	The weight of Northern Wheatgrass shoot after drying at 60 - 70 degrees C
y_{15}	Alf root length	The root length of Alfalfa
y_{16}	Alf root dry mass	The weight of Alfalfa root after drying at 60 - 70 degrees C
y_{17}	Alf shoot length	The shoot length of Alfalfa
y_{18}	Alf shoot dry mass	The weight of Alfalfa shoot after drying at 60 - 70 degrees C

Table 4.2: Desciption of variables - contamination levels and soil properties.

Notation	Variable Name	Description
y_{19}	F2	The PHC fraction F2 concentration in the soil
y_{20}	F3	The PHC fraction F3 concentration in the soil
y_{21}	F4	The PHC fraction F4 concentration in the soil
y_{22}	Total PHC	The Total PHC concentration (sum of F2, F3 & F4) in the soil
y_{23}	Silt	The % of Silt in the soil
y_{24}	Clay	The % of Clay in the soil
y_{25}	Total Nitrogen	The % of Total Nitrogen in the soil
y_{26}	Total Carbon	The % of Total Carbon in the soil
y_{27}	Organic Matter	The % of Organic Matter in the soil
y_{28}	pH	The pH level in the soil
y_{29}	Phosphorous	The Phosphorus content in the soil
y_{30}	WHC	The Water-Holding Capacity of the soil

4.2 Software implementation

SPSS (version 23) was used to calculate univariate statistics, including bivariate correlations between the study variables (Table 4.3). The mean values represent the average of a variable across the sample dataset while the standard deviation is a measure of how widely a variable is spread across the dataset. The bivariate correlations provide the measure of association of one variable to another variable; a negative correlation value shows that as one variable increases, the other decreases while a positive correlation value indicates that as one variable increases (or decreases), the other also increases (or decreases). The PHC concentration fraction F2 and the total PHC concentration variables were log + 1 transformed because they had values covering a wide range of concentrations and so some values were much higher than others. These variables had particularly high standard deviations (7491.91 and 7511.55 respectively) and high correlation (0.99) with each other. High mulicollinearity may increase the standard errors of some coefficients causing some estimates to be unstable and resulting in some of the variables being insignificant. Standardizing highly correlated variables like the total PHC concentration variable and the F2 variable provided a means to reduce multicollinearity and mitigate its associated problems. The SEM analysis procedures were carried out using the "lavaan" package in R statistical software (R Core Team, 2013; Rosseel, 2012) (see Appendix B for all software implementations). The method of Maximum Likelihood (ML) estimation, which is the default method of estimation in Structural Equation Modeling in most SEM computer programs, was used in analysis. Distributions were fit using the "fitdistr" package (Delignette-Muller, & Dutang, 2014), while non-linear regressions were carried out using the "drc" package (Ritz & Streibig, 2005) in R.

4.3 Results

4.3.1 Species Sensitivity Distribution (SSD): cross-site analysis

Species sensitivity distributions (SSDs) are one of the current methods for assessing toxicological responses and developing soil remediation guidelines. The SSD shows the variation in

Table 4.3: Univariate statistics and bivariate correlations.
M - Mean, SD - Standard deviation.

	M	SD	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}	y_{14}	y_{15}	y_{16}	y_{17}	y_{18}
y_2	22.32	29.09	1.00																
y_3	24.93	29.71	0.04	1.00															
y_4	21.22	34.26	0.06	-0.01	1.00														
y_5	366.00	400.03	0.43	-0.10	-0.18	1.00													
y_6	26.53	35.32	0.46	0.23	0.14	0.62	1.00												
y_7	297.85	360.69	0.69	-0.19	0.95	0.68	0.65	1.00											
y_8	28.57	17.47	0.64	0.53	0.59	0.41	0.18	0.81	1.00										
y_9	239.08	316.57	-0.09	0.72	0.04	-0.10	0.59	-0.15	0.70	1.00									
y_{10}	71.27	51.43	0.65	0.53	0.71	0.20	0.07	0.68	0.86	0.48	1.00								
y_{11}	97.03	64.21	0.56	0.41	0.30	0.30	0.55	0.91	0.84	0.28	0.74	1.00							
y_{12}	30.35	55.89	-0.17	0.42	0.73	-0.28	0.05	0.89	0.88	0.56	0.93	0.09	1.00						
y_{13}	108.58	58.00	0.39	0.55	0.37	0.64	0.49	0.92	0.80	0.94	0.59	0.66	0.24	1.00					
y_{14}	39.20	47.96	0.11	0.42	0.79	-0.33	-0.08	0.87	0.63	0.45	0.87	0.56	0.69	0.05	1.00				
y_{15}	103.25	86.07	0.50	0.56	-0.46	0.77	0.68	-0.30	0.67	0.90	0.46	0.84	-0.46	0.92	-0.37	1.00			
y_{16}	6.81	5.24	0.78	0.54	0.55	0.54	0.53	0.86	0.76	0.69	0.78	0.87	0.74	0.72	0.61	0.76	1.00		
y_{17}	41.02	37.11	0.65	0.11	-0.43	0.77	0.73	-0.25	0.67	-0.01	0.54	0.79	-0.48	0.85	-0.40	0.94	0.85	1.00	
y_{18}	23.00	31.78	0.77	0.37	0.69	0.16	0.16	0.53	0.65	0.19	0.87	0.60	0.76	0.29	0.92	0.27	0.73	0.43	1.00
	M	**SD**	y_{19}	y_{20}	y_{21}	y_{22}	y_{23}	y_{24}	y_{25}	y_{26}	y_{27}	y_{28}	y_{29}	y_{30}					
y_{19}	3221.42	7491.91	1.00																
y_{20}	445.83	834.64	-0.14	1.00															
y_{21}	397.27	910.53	-0.22	0.99	1.00														
y_{22}	3645.50	7511.77	0.99	-0.03	-0.12	1.00													
y_{23}	34.80	13.18	0.24	-0.75	-0.76	0.16	1.00												
y_{24}	26.78	9.14	0.24	-0.75	-0.76	0.19	-0.47	1.00											
y_{25}	17.50	43.18	-0.07	-0.17	-0.15	-0.09	-0.82	0.77	1.00										
y_{26}	20.40	46.05	0.20	-0.16	-0.17	0.19	0.81	-0.44	0.28	1.00									
y_{27}	8.50	5.65	-0.02	-0.08	-0.08	-0.04	0.48	0.32	-0.38	-0.49	1.00								
y_{28}	10.64	12.84	-0.10	-0.01	-0.06	-0.11	-0.87	0.40	0.04	-0.28	0.87	1.00							
y_{29}	26.05	25.38	-0.16	0.77	0.73	-0.06	-0.06	-0.59	-0.40	-0.28	-0.04	-0.20	1.00						
y_{30}	52.83	44.89	0.04	-0.34	-0.25	-0.01	0.64	0.45	-0.26	-0.37	0.93	0.65	-0.26	1.00					

sensitivity of the species to the PHC concentrations. Following current methods, SSDs were developed for the two sites used in this research for cross-site analysis. This process involved the utility of nonlinear procedures for quantifying the relationships between total PHC concentrations and the species endpoints to estimate IC_{25} values. The endpoint variables are described in Table 4.4.

The total PHC concentration variable was log + 1 transformed to make the data easier to handle and interpret. Most sets of results in toxicology can be fit satisfactorily using either linear, logistic, exponential or Gompertz distributions (EC, 2005). Linear methods are relatively simple, and there may be cases where they are not sufficient to capture a complex relationship like that of PHC contaminant concentration to response (EC, 2005). Different models (three- and four- parameter log-logistic models, three- and four- parameter weibull models, five-parameter baroreflex model, and three-parameter exponential decay model) were fit in R software (R Core Team, 2013) using the "drm" function of the "drc" package (Ritz & Streibig, 2005) (see Table 4.5). The mathematical formula for each of the models are given below (Ritz & Strebig, 2015).

1. The three-parameter log-logistic model (LL (3)) allows specification of a three-parameter log-logistic function with the lower limit at zero.

$$f(x) = 0 + \frac{d - 0}{1 + exp(b(log(x) - log(e)))} \tag{4.1}$$

Table 4.4: Description of variables.

Variable Name	Description
EA progeny	Number of fertile progeny produced by Eisenia andrei
EA progeny wet mass	The weight of Eisenia andrei progeny before drying
EA progeny dry mass	The weight of Eisenia andrei progeny after drying at 60 - 70 degrees C
FC progeny	Number of fertile progeny produced by Folsomia candida
FC survival	% of Folsomia candida that survived after tests were done
Ba root length	The root length of Barley
Ba root dry mass	The weight of Barley root after drying at 60 - 70 degrees C
Ba shoot length	The shoot length of Barley
Ba shoot dry mass	The weight of Barley shoot after drying at 60 - 70 degrees C
NWG root length	The root length of Northern Wheatgrass
NWG root dry mass	The weight of Northern Wheatgrass root after drying at 60 - 70 degrees C
NWG shoot length	The shoot length of Northern Wheatgrass
NWG shoot dry mass	The weight of Northern Wheatgrass shoot after drying at 60 - 70 degrees C
Alf root length	The root length of Alfalfa
Alf root dry mass	The weight of Alfalfa root after drying at 60 - 70 degrees C
Alf shoot length	The shoot length of Alfalfa
Alf shoot dry mass	The weight of Alfalfa shoot after drying at 60 - 70 degrees C
F2	The PHC fraction F2 concentration in the soil
F3	The PHC fraction F3 concentration in the soil
F4	The PHC fraction F4 concentration in the soil
Total PHC	The Total PHC concentration (sum of F2, F3 & F4) in the soil

2. The four-parameter log-logistic model (LL (4)) presents a four-parameter log-logistic function and is written mathematically as:

$$f(x) = c + \frac{d - c}{1 + exp(b(log(x) - log(e)))} \tag{4.2}$$

e is called the inflection point at which the function is symmetric.

3. The three-parameter weibull model (weibull (3)) provides a three-parameter weibull function and is written mathematically as:

$$f(x) = 0 + (d - 0)exp(-exp(b(log(x) - e))) \tag{4.3}$$

where *exp(e)* is called the inflection point at which the function is asymmetric.

4. The four-parameter weibull model (weibull (4)) as the name implies provides a weibull function with four parameters. A weibull (4) model is written as:

$$f(x) = c + (d - c)exp(-exp(b(log(x) - log(e)))) \tag{4.4}$$

where *exp(e)* is called the inflection point at which the function is asymmetric.

5. The five-parameter baroreflex (baro (5)) is a baroreflex dose-response function with five parameters. It is represented mathematically by the formula:

$$f(x) = c + \frac{d - c}{1 + fexp(b_1(log(x) - log(e))) + (1 - f)exp(b_2(log(x) - log(e)))} \tag{4.5}$$

where:

$$f = \frac{1}{1 + exp((2b_1b_2|b_1 + b_2|)(log(x) - log(e)))} \tag{4.6}$$

If b_1 is not equal in value to b_2, then the function is asymmetric.

6. The mean function of a three-parameter exponential decay model (Exp (3)) can be written as:

$$f(x) = c + (d - c)exp(-x/e)) \tag{4.7}$$

The best model for each endpoint was chosen by comparing AICs and the corresponding IC_{25} values were estimated using the "ED" function in the "drc" package. Figures 4.1 and

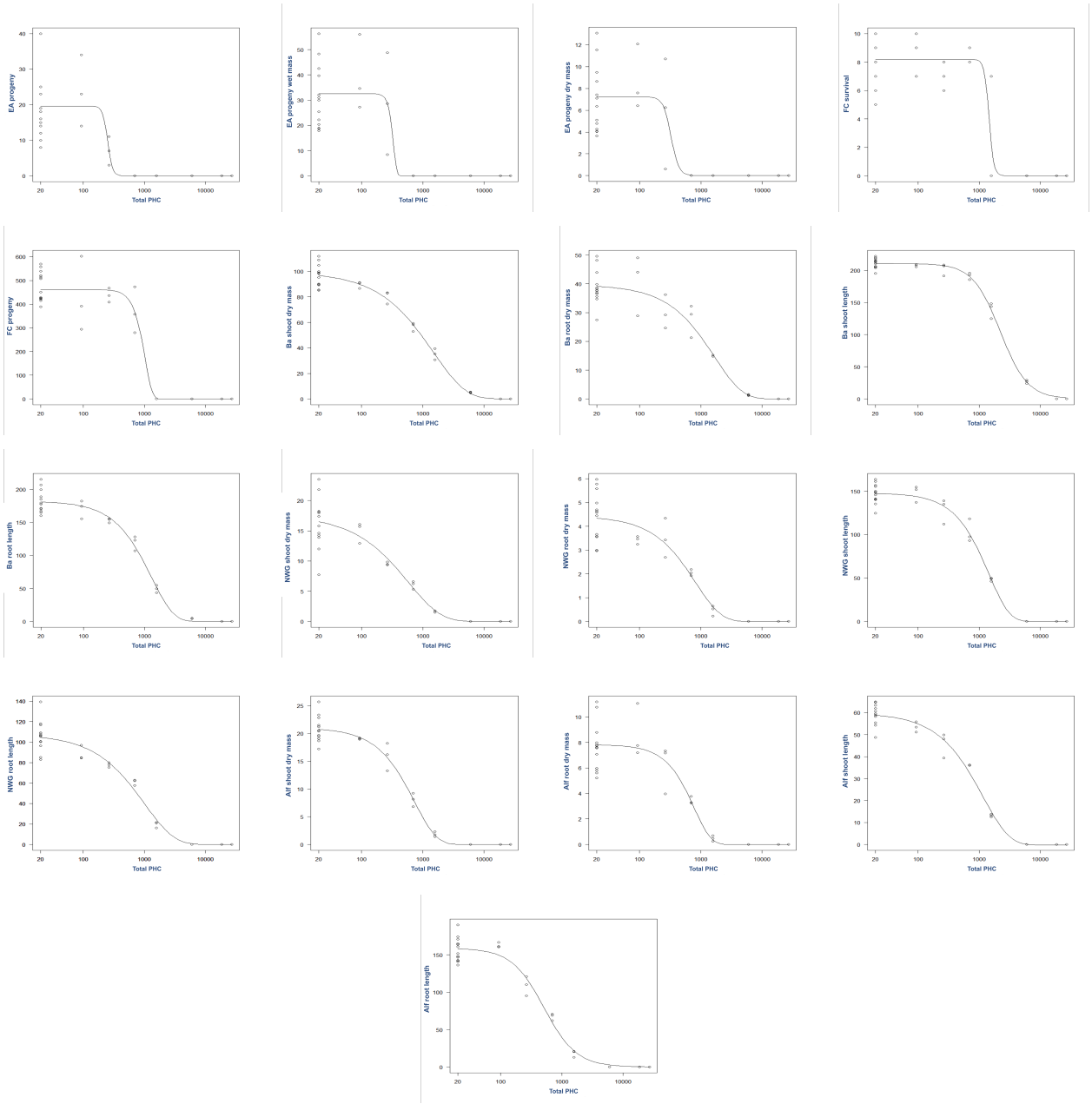


Figure 4.1: Distribution of endpoints in site 1 (PHC was log + 1 transformed).

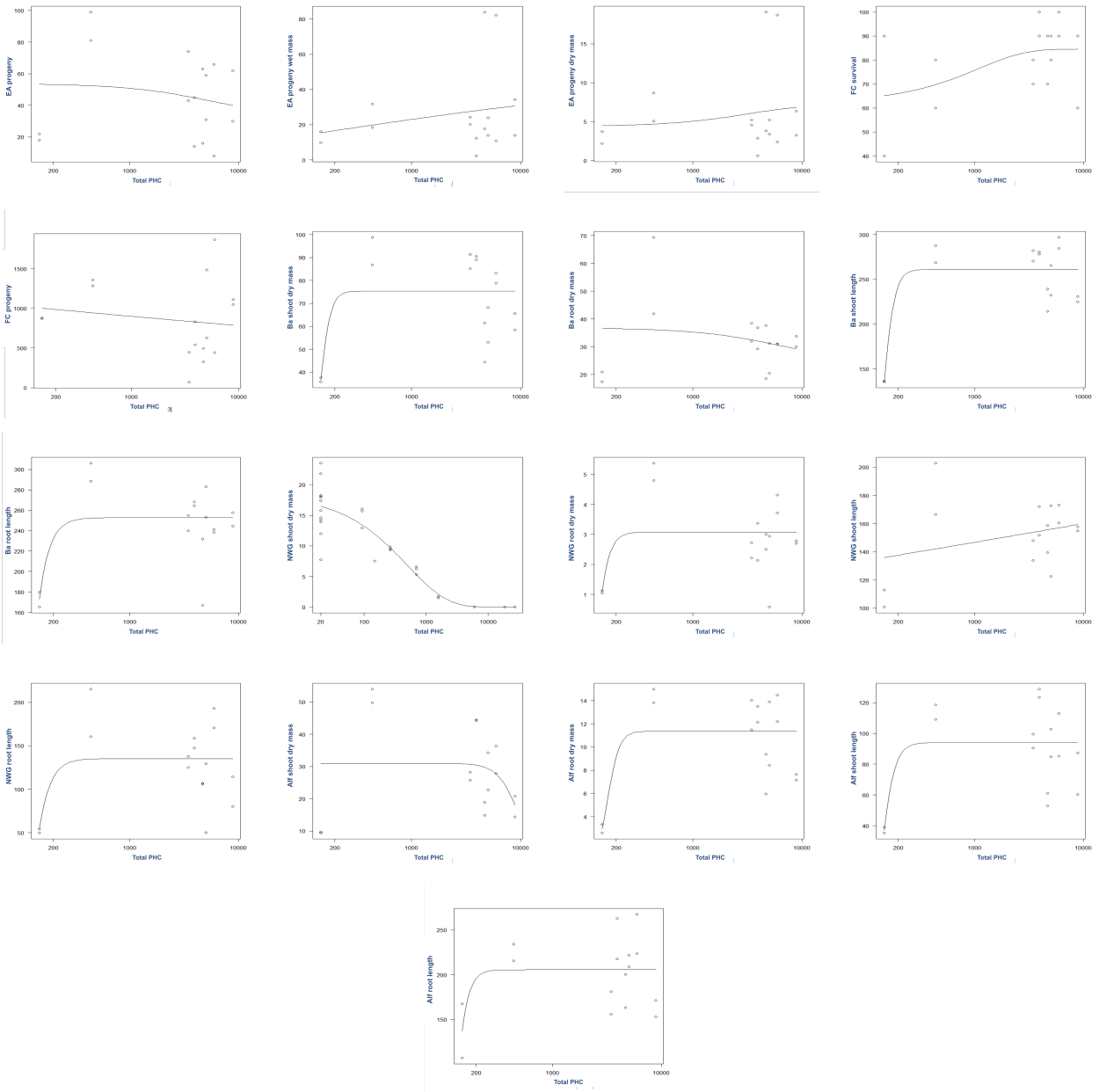


Figure 4.2: Distribution of endpoints in site 2 (PHC was log + 1 transformed).

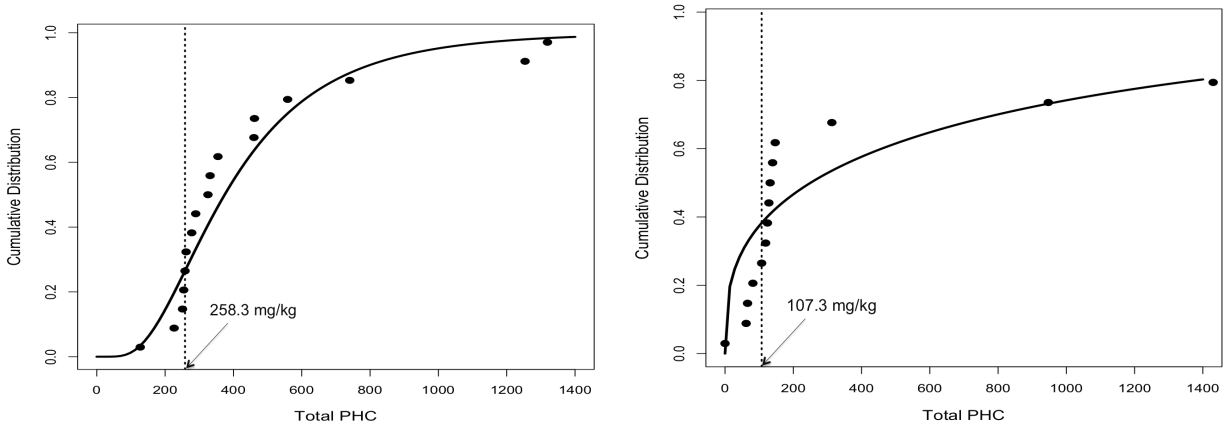
Table 4.5: AICs for the models fit for the endpoint and the corresponding IC_{25} values (mg/kg dry soil) for the best model for each endpoint.
LL(3) - three-parameter log-logistic model; LL(4) - four-parameter log-logistic model; Exp (3) - three-parameter exponential decay model; Weibull (3) - three-parameter weibull model; Weibull (4) - four-parameter weibull model; Baro (5) - five-parameter baroreflex model.

Site 1								
	LL (3)	LL (4)	EXD (3)	Weibull (3)	Weibull (4)	Baro (5)	Best Model	IC25 (SE)
EA progeny	223.4	225.4	228.3	223.4	225.4	225.6	LL(3)	226.4 (191.4)
EA progeny wet mass	259.8	261.8	265.9	259.8	261.8	262.0	Weibull(3)	289.6 (310.5)
EA progeny dry mass	162.8	164.8	167.4	162.8	164.8	166.8	LL(3)	277.9 (68.7)
FC survival	133.6	135.6	141.1	133.6	135.6	137.6	LL(3)	1319.3 (1446.7)
FC progeny	383.2	385.2	400.1	383.2	385.1	386.3	Weibull(3)	740.5 (87.5)
Ba shoot dry mass	222.5	223.1	222.6	220.3	222.3	224.5	Weibull(3)	325.4 (62.1)
Ba root dry mass	214.3	216.0	213.4	213.3	215.3	217.3	Weibull(3)	460.0 (157.5)
Ba shoot length	231.3	232.5	248.1	232.7	234.7	234.2	LL(3)	1253.7 (58.9)
Ba root length	271.7	273.6	271.0	269.5	271.4	271.7	Weibull(3)	461.5 (71.2)
NWG shoot dry mass	167.2	169.1	167.5	167.1	169.1	171.1	Weibull(3)	127.2 (77.3)
NWG root dry mass	78.3	80.1	76.9	76.9	78.9	79.1	Weibull(3)	251.0 (121.1)
NWG shoot length	253.2	255.1	254.4	250.6	252.5	256.1	Weibull(3)	558.7 (68.5)
NWG root length	261.8	262.9	258.3	258.1	260.0	258.2	Weibull(3)	254.5 (86.0)
Alf shoot dry mass	133.2	135.0	133.4	131.8	133.8	135.7	Weibull(3)	258.3 (43.6)
Alf root dry mass	124.5	126.5	126.8	124.5	126.5	128.5	Weibull(3)	354.7 (99.2)
Alf shoot length	193.3	194.1	186.6	186.6	188.6	186.0	Exp(3)	331.7 (26.3)
Alf root length	271.0	272.9	272.4	271.6	273.6	274.9	LL(3)	261.4 (35.3)
Site 2								
	LL (3)	LL (4)	Exp (3)	Weibull (3)	Weibull (4)	Baro (5)	Best Model	IC25 (SE)
EA progeny	158.1	160.1	157.7	158.1	160.1	160.6	Exp(3)	1430.2 (6008.1)
EA progeny wet mass	152.8	154.8	152.8	152.8	152.8	156.8	Weibull(3)	61.9(1741.5)
EA progeny dry mass	105.8	107.8	105.9	105.5	107.5	109.4	Weibull(3)	947.2(4042.7)
FC survival	138.1	139.9	137.8	138.1	139.8	141.9	Exp(3)	313.0 (695.9)
FC progeny	249.7	251.6	250.0	249.7	250.5	251.5	LL(3)	4.5e-05 (1.1e-03)
Ba shoot dry mass	140.1	142.1	148.8	148.0	149.9	144.1	LL(3)	132.2 (72.5)
Ba root dry mass	132.0	134.0	131.9	132 .5	133.2	136.0	Exp(3)	1658.7 (6530.3)
Ba shoot length	155.7	157.7	155.7	155.7	157.7	159.7	LL(3)	128.5 (56.1)
Ba root length	170.1	163.9	171.2	171.2	172.1	165.8	LL(4)	66.0(563.8)
NWG shoot dry mass	174.7	176.6	175.8	174.3	176.3	178.6	Weibull (3)	81.6 (68.9)
NWG root dry mass	61.0	63.0	61.1	61.0	63.0	64.9	LL(5)	119.0 (172.1)
NWG shoot length	154.3	-	156.0	154.3	156.3	149.8	Weibull(3)	1960.2 (15204.0)
NWG root length	176.9	-	177.3	176.9	178.9	175.0	LL(5)	107.3 (35.8)
Alf shoot dry mass	135.6	139.1	136.3	137.1	139.1	122.7	LL(3)	7507.2 (3680.6)
Alf root dry mass	85.9	87.9	-	-	97.9	89.9	LL(3)	147.0 (18.0)
Alf shoot length	-	153.3	151.8	151.8	151.8	153.8	Weibull(3)	139.4 (21.2)
Alf root length	167.0	169.0	167.0	167.0	169.0	169.0	Weibull(3)	124.3 (90.1)

4.2 show a graphical representation of the best model for each endpoint for site 1 and 2 respectively.

Lognormal, exponential decay and gamma distributions were fit to the estimated IC_{25} values using the "fitdist" function in the "fitdistplus" package of R software (Delignette-Muller, & Dutang, 2014). For site 1, the AIC values for the lognormal, exponential and gamma distributions fit to the IC_{25}s were 235.84, 244.16 and 238.98 respectively. The lognormal distribution had an AIC value significantly different (i.e. greater than 2.0) from the AIC values of the exponential and gamma distributions and so the cdfcomp function was used to plot the lognormal cumulative distribution of the IC_{25} values against the total PHC concentration (see Figure 4.3a). For site 2, the gamma distribution had an AIC value significantly different (i.e. greater than 2.0) from the AIC values of the lognormal, and exponential distributions fit for the IC_{25} values (lognormal AIC = 259.68, exponential AIC = 266.43, gamma AIC = 245.35) and so the cdfcomp function was used to plot the gamma cumulative distribution of

the IC_{25} values against the total PHC concentration (Figure 4.3b). A single IC_{25} was estimated from each of these cumulative IC_{25} distributions for the individual sites. These single IC_{25} values are calculated by current methods as the remediation guidelines. Therefore, the remediation guidelines were estimated as 258.3mg/kg for site 1 and 107.3mg/kg for site 2.



(a) Site 1 - Cumulative distribution of IC_{25} values against contaminant concentration. The curved line is the fitted lognormal distribution; the dotted vertical lines indicate the 25th percentile concentrations of the cumulative lognormal distribution.

(b) Site 2 - Cumulative distribution of IC_{25} values against contaminant concentration. The curved line is the fitted gamma distribution; the dotted vertical lines indicate the 25th percentile concentrations of the cumulative lognormal distribution.

Figure 4.3: Species Sensitivity Distributions (SSDs) for individual sites.

As previously stated, this is the current method employed by toxicologists to estimate IC_{25} values for remediation purposes. The use of this method is, however, questionable not only because it derives a single IC_{25} estimate based on other IC_{25} estimates but also because some species are overrepresented in the analysis. We demonstrate in the following sections an alternative method to the SSD method. This alternative method carried out in SEM provides a way to directly analyze species responses to create single site-level IC_{25} estimates.

4.3.2 Confirmatory Factor Analysis

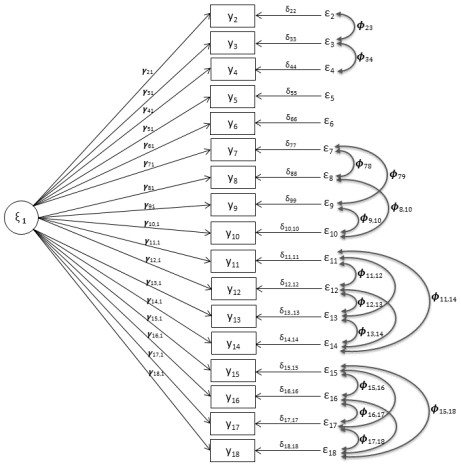
CFA was used to examine the extents of the interrelationships and covariations (if any), between the observed and latent variables. In this process, factor loadings and unique variances were estimated to influence the choice of the best indicators for the latent variables.

Table 4.6: Description of variables.

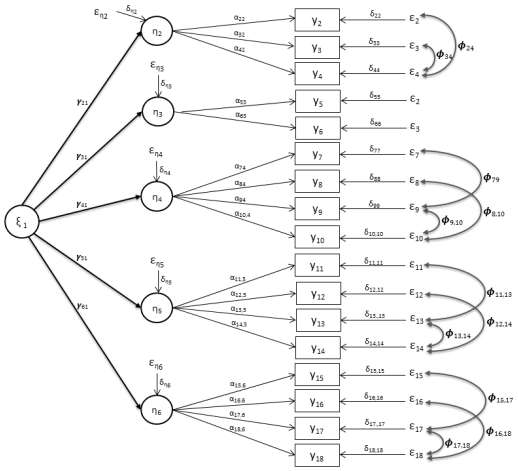
Notation	Variable Name	Variable Type	Description
y_2	EA progeny No.	Manifest endogenous	Number of fertile progeny produced by Eisenia andrei
y_3	EA progeny wet mass	Manifest endogenous	The weight of Eisenia andrei progeny before drying
y_4	EA progeny dry mass.	Manifest endogenous	The weight of Eisenia andrei progeny after drying at 60 - 70 degrees C
y_5	FC progeny No.	Manifest endogenous	Number of fertile progeny produced by Folsomia candida
y_6	FC survival.	Manifest endogenous	% of Folsomia candida that survived after tests were done
y_7	Ba root length	Manifest endogenous	The root length of Barley
y_8	Ba root dry mass	Manifest endogenous	The weight of Barley root after drying at 60 - 70 degrees C
y_9	Ba shoot length	Manifest endogenous	The shoot length of Barley
y_{10}	Ba shoot dry mass	Manifest endogenous	The weight of Barley shoot after drying at 60 - 70 degrees C
y_{11}	NWG root length	Manifest endogenous	The root length of Northern Wheatgrass
y_{12}	NWG root dry mass	Manifest endogenous	The weight of Northern Wheatgrass root after drying at 60 - 70 degrees C
y_{13}	NWG shoot length	Manifest endogenous	The shoot length of Northern Wheatgrass
y_{14}	NWG shoot dry mass	Manifest endogenous	The weight of Northern Wheatgrass shoot after drying at 60 - 70 degrees C
y_{15}	Alf root length	Manifest endogenous	The root length of Alfalfa
y_{16}	Alf root dry mass	Manifest endogenous	The weight of Alfalfa root after drying at 60 - 70 degrees C
y_{17}	Alf shoot length	Manifest endogenous	The shoot length of Alfalfa
y_{18}	Alf shoot dry mass	Manifest endogenous	The weight of Alfalfa shoot after drying at 60 - 70 degrees C
ξ_1	Aggregate Response	Latent exogenous	The latent variable representing aggregate species response
η_2	EA Response	Latent endogenous	The latent variable representing Eisenia andrei response
η_3	FC Response	Latent endogenous	The latent variable representing Folsomia candida response
η_4	Ba Response	Latent endogenous	The latent variable representing Barley response
η_5	NWG Response	Latent endogenous	The latent variable representing Northern Wheatgrass response
η_6	Alf Response	Latent endogenous	The latent variable representing Alfalfa response
δ		Estimates	Path coefficients from disturbance to an endogenous variable
α		Estimates	Path coefficients from an endogenous variable to another endogenous variable
γ		Estimates	Path coefficients from an exogenous variable to an endogenous variable
ϵ		Estimates	Disturbance/error for endogenous variables

Indicators with factor loadings that were significant at 5% level of significance were retained in the model. The models were initially developed based on theoretical knowledge using confirmatory factor analysis. All observed variables were standardized before each model in this section was analyzed. Standardization was achieved by setting the means and variances of the observed variables to zero and unity respectively. The path diagrams for each model specified are shown in Figure 4.4 and the variable notations are in Table 4.6.

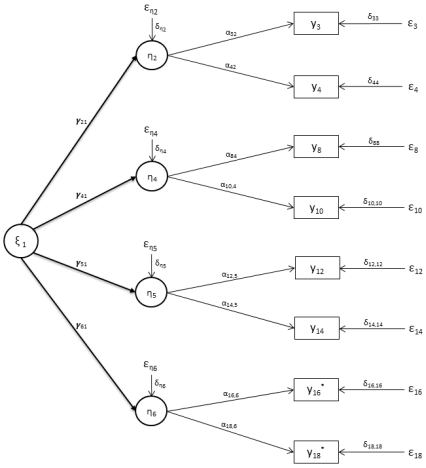
These models were used to combine multiple species endpoints into a single latent variable representing the responses of the species to the PHC contaminant concentrations. Model 1 (Figure 4.4a) was a first-order latent variable model where all the species endpoints (y_2 to y_{18}) were combined into a single aggregate response (ξ_1) and y_2 to y_{18} are referred to as indicators of the latent ξ_1 . Models 2 and 3 (Figures 4.4b & 4.4c respectively) were second-order latent



(a) model 1



(b) model 2



(c) model 3: * is used for variables that have been log+1 transformed

Figure 4.4: Models 1, 2 & 3 combining multiple species endpoints into a single latent variable ξ_1, representing the responses of the species to the PHC contaminant concentrations.

Table 4.7: Model summaries 1 - fit indices for models 1, 2 & 3.
CFI - comparative fit index; TLI- tucker-lewis index; AIC - akaike's information criterion; RMSEA - root mean square error of approximation; SRMR - standardized root mean square residual.

	Model 1 (n=50)	**Model 2 (n=50)**	**Model 3 (n=50)**
χ^2	${\chi^2}_{101} = 406.76; p<0.001$	${\chi^2}_{103} = 408.075; p<0.001$	${\chi^2}_{16} = 21.56; p = 0.16$
CFI	0.83	0.83	0.99
TLI	0.77	0.78	0.99
AIC	967.50	964.82	377.38
RMSEA	0.25; 95% CI 0.22 - 0.27	0.24; 95% CI 0.22 - 0.27	0.083; 95% CI 0.000 - 0.17
SRMR	0.10	0.12	0.049

variable models. Model 2 first created first order latent variables (η_2 to η_6) by taking into account that there might exist separate responses across each species and then combined these first order latent variables into an aggregate response latent variable (ξ_1). Model 2 was however of concern because the root and shoot length variables of Barley and Northern Wheatgrass had negative variance. Model 3 was specified by also creating first order latent variables (η_2, η_4, η_5 and η_6) taking into account only mass measurements across the species and then combined these latent variables into an aggregate response latent variable (ξ_1). Non-linear relationships between two endpoints (Alfalfa root dry mass and Alfalfa shoot dry mass) and the predicted aggregate response in model 3 were linearized by performing log+1 transformations.

The three models were compared based on the fit indices specified in section 3.1.4. From Table 4.7, it can be seen that model 3 described the data well and was a much better fit than either models 1 and 2, based on the fact that it had a non-significant chi-square value ($\chi^2(16, N = 50) = 21.56$, p = 0.16), the highest CFI and TLI of 0.99 and 0.99 respectively, the lowest RMSEA value (RMSEA = 0.083; 95% CI 0.00 - 0.17) and the lowest SRMR value (0.049). The RMSEA should ideally be less than 0.05, but it is important to note that RMSEA is likely to over-reject true models when we have smaller sample sizes. The AIC is an appropriate method for comparing the three models since the models are from the same dataset, and the model 3 had an AIC value significantly different (i.e. greater than 2.0) from the AIC values of the other models. This showed that model 3 gave the best representation of the data compared to the other two models.

Using the common LISREL (Linear Structural Equations), each manifest endogenous variable in model 3 can be represented by the following system of linear equations:

$$y_3 = \alpha_{32}\eta_2 + \delta_{33}\epsilon_3$$

$$y_4 = \alpha_{42}\eta_2 + \delta_{44}\epsilon_4$$

$$y_8 = \alpha_{8,4}\eta_4 + \delta_{88}\epsilon_8$$

$$y_{10} = \alpha_{10,4}\eta_4 + \delta_{10,10}\epsilon_{10}$$

$$y_{12} = \alpha_{12,5}\eta_5 + \delta_{12,12}\epsilon_{12}$$

$$y_{14} = \alpha_{14,5}\eta_5 + \delta_{14,14}\epsilon_{14}$$

$$y_{16} = \alpha_{16,6}\eta_6 + \delta_{16,16}\epsilon_{16}$$

$$y_{18} = \alpha_{18,6}\eta_6 + \delta_{18,18}\epsilon_{18}$$

and each latent endogenous vaiable in can be represented by:

$$\eta_2 = \gamma_{21}\xi_1 + \delta_{\eta_2}\epsilon_{\eta_2}$$

$$\eta_4 = \gamma_{41}\xi_1 + \delta_{\eta_4}\epsilon_{\eta_4}$$

$$\eta_5 = \gamma_{51}\xi_1 + \delta_{\eta_5}\epsilon_{\eta_5}$$

$$\eta_6 = \gamma_{61}\xi_1 + \delta_{\eta_6}\epsilon_{\eta_6}$$

The vector-matrix notation of the above system of linear equations can be expressed as follows:

$$\begin{bmatrix} \eta \\ y \end{bmatrix} = \mathbb{A} \begin{bmatrix} \eta \\ y \end{bmatrix} + \mathbb{G} \begin{bmatrix} \xi \\ x \end{bmatrix} + \begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} \tag{4.8}$$

where;

$$\mathbb{A}\begin{bmatrix}\eta\\ y\end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & 0 & 0 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \dots & \vdots \\ \alpha_{32} & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{42} & 0 & 0 & 0 & 0 & \dots & 0 \\ 0 & \alpha_{84} & 0 & 0 & 0 & \dots & 0 \\ 0 & \alpha_{10,4} & 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 1 & 0 & 0 & \dots & 0 \\ 0 & 0 & \alpha_{14,5} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & 1 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{18,6} & 0 & \dots & 0 \end{bmatrix}_{12\times 12} \begin{bmatrix} \eta_2 \\ \eta_4 \\ \eta_5 \\ \eta_6 \\ y_3 \\ y_4 \\ y_8 \\ y_{10} \\ y_{12} \\ y_{14} \\ y_{16}* \\ y_{18}* \end{bmatrix}_{12\times 1}, \tag{4.9}$$

$$\eta = \begin{bmatrix}\eta_2\\ \eta_4\\ \eta_5\\ \eta_6\end{bmatrix}_{4\times 1}, y = \begin{bmatrix} y_3 \\ y_4 \\ y_8 \\ y_{10} \\ y_{12} \\ y_{14} \\ y_{16}* \\ y_{18}* \end{bmatrix}_{8\times 1}, \begin{bmatrix}\eta\\ y\end{bmatrix}_{12\times 1} \tag{4.10}$$

;

$$\mathbb{G}\begin{bmatrix}\xi\\ x\end{bmatrix} = \begin{bmatrix} \gamma_{21} \\ \gamma_{41} \\ \gamma_{51} \\ \gamma_{61} \\ 0 \\ \vdots \\ 0 \end{bmatrix}_{12\times 1} \begin{bmatrix}\xi_1\end{bmatrix}_{1\times 1} \tag{4.11}$$

(the matrix $\begin{bmatrix} \xi \\ x \end{bmatrix}$ only contains ξ because there are no manifest exogenous variables x in the model) ;

$$\begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} = \begin{bmatrix} \Delta_{4\times4} & 0_{4\times8} \\ 0_{8\times4} & \Psi_{8\times8} \end{bmatrix}_{12\times12} \begin{bmatrix} \zeta_{4\times1} \\ \epsilon_{8\times1} \end{bmatrix}_{12\times1} = \tag{4.12}$$

$$\begin{bmatrix} \begin{pmatrix} \delta_{\eta2} & 0 & 0 & 0 \\ 0 & \delta_{\eta4} & 0 & 0 \\ 0 & 0 & \delta_{\eta5} & 0 \\ 0 & 0 & 0 & \delta_{\eta6} \end{pmatrix} & \begin{pmatrix} 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \dots & 0 \end{pmatrix} \\ \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} & \begin{pmatrix} \delta_{33} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \delta_{44} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \delta_{88} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \delta_{10,10} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \delta_{12,12} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \delta_{14,14} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \delta_{16,16} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \delta_{18,18} \end{pmatrix} \end{bmatrix}_{12\times12} \begin{bmatrix} \begin{pmatrix} \epsilon_{\eta2} \\ \epsilon_{\eta4} \\ \epsilon_{\eta5} \\ \epsilon_{\eta6} \end{pmatrix} \\ \begin{pmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \\ \epsilon_{16} \\ \epsilon_{18} \end{pmatrix} \end{bmatrix}_{12\times1} \tag{4.13}$$

,

$$\zeta = \begin{bmatrix} \epsilon_{\eta2} \\ \epsilon_{\eta4} \\ \epsilon_{\eta5} \\ \epsilon_{\eta6} \end{bmatrix}_{4\times1} , \epsilon = \begin{bmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \\ \epsilon_{16} \\ \epsilon_{18} \end{bmatrix}_{8\times1} \tag{4.14}$$

Inspection of the full results of the analysis of model 3 showed that the path coefficients

were highly significant with all the p values less than 0.001 (Table 4.8). All species responses were significant indicators of the overall aggregate response. Higher values of path coefficients indicate stronger relationships. *Eisenia andrei* progeny dry mass had a path coefficient of 0.98 on the total *Eisenia andrei* mass response, and the total *Eisenia andrei* species response explained 98% of the *Eisenia andrei* progeny dry mass variance. Barley root dry mass had a path coefficient of 0.93 on the Barley species mass response, and the total Barley species response explained 87% of the Barley shoot dry mass variance. Northern Wheatgrass root dry mass also had a high coefficient of 0.95 and the total Northern Wheatgrass species response explained 92% of the Northern Wheatgrass shoot dry mass variance. Finally, the path coefficient of the natural-log + 1 transformed Alfalfa root dry mass was 0.97, with the total Alfalfa species response explaining 95% of the variance of the Alfalfa root dry mass variable.

To summarize the results, there is a 0.99 and 0.98 unit change in the wet mass and dry mass respectively of *Eisenia andrei* per standard deviation change in the total *Eisenia andrei* response; a 0.93 and 0.96 unit change in the root dry mass and shoot dry mass respectively of Barley per standard deviation change in the total Barley response; a 0.95 unit change in both the root dry mass and shoot dry mass of Northern Wheatgrass per standard deviation change in the total Northern Wheatgrass response; and a 0.97 and almost a unit change in the root dry mass and shoot dry mass respectively of Alfalfa per standard deviation change in the total Alfalfa response. Also, for every 1 unit change in standard deviation in the aggregate response, there is a 0.52, 1, 0.94, 0.91 unit change in *Eisenia andrei*, Barley, Northern Wheatgrass and Alfalfa species responses respectively.

The aggregate response, *Eisenia andrei* response, Barley response, Northern Wheatgrass response and Alfalfa response latent variables in model 3 were predicted across the dataset using the predict function in lavaan. The scatterplot of the predicted aggregate response variable against the predicted *Eisenia andrei* response, Barley response, Northern Wheatgrass response and Alfalfa sresponse latent variables and the scatterplot of the predicted *Eisenia andrei* response, Barley response, Northern Wheatgrass response and Alfalfa response latent variables against their indicators demonstrate are shown in Figures 4.5 and 4.6 respectively.

Table 4.8: Full results for Model 3 including path coefficients and their standard errors (observed variables standardized - col. 2), test of path significance (col. 3), confidence intervals (col. 4), and path coefficients (latent and observed variables standardized - col. 5).

Path	Estimate(Standard error)	P value	Lower CI - Upper CI	Standardized estimates
Latent Variables				
Aggregate response → $EAresponse(\gamma_{21})$	1.00			0.52
Aggregate response → $Baresponse(\gamma_{41})$	1.84(0.43)	<0.001	0.99 - 2.69	1.03
Aggregate response → $NWGresponse(\gamma_{51})$	1.72(0.41)	<0.001	0.92 - 2.53	0.94
Aggregate Response → $Alfresponse(\gamma_{61})$	1.69(0.41)	<0.001	0.89 - 2.49	0.91
EA response → $EAprogenywetmass(\alpha_{32})$	1.00			0.99
EA response → $EAprogenydrymass(\alpha_{42})$	0.99(0.025)	<0.001	0.94 - 1.04	0.98
Ba response → $Barootdrymass(\alpha_{84})$	1.00			0.93
Ba response → $Bashootdrymass(\alpha_{10,4})$	1.04(0.068)	<0.001	0.91 - 1.17	0.96
NWG response → $NWGrootdrymass(\alpha_{12,5})$	1.00			0.95
NWG response → $NWGshootdrymass(\alpha_{14,5})$	1.00(0.063)	<0.001	0.87 - 1.12	0.95
Alf response → $Alfrootdrymass(\alpha_{16,6})$	1.00			0.97
Alf response → $Alfshootdrymass(\alpha_{18,6})$	1.03(0.031)	<0.001	0.97 - 1.09	1.01
Variances				
Aggregate response	0.27(0.13)			1.00
EA response (δ_{η_2})	0.72(0.15)			0.73
Ba response (δ_{η_4})	-0.049(0.024)			-0.058
NWG response (δ_{η_5})	0.11(0.037)			0.12
Alf response (δ_{η_6})	0.17(0.040)			0.18
EA progeny wet mass (δ_{33})	-0.009(0.021)			-0.009
EA progeny dry mass (δ_{44})	0.017(0.021)			0.017
Ba root dry mass (δ_{88})	0.12(0.029)			0.13
Ba shoot dry mass ($\delta_{10,10}$)	0.055(0.020)			0.057
NWG root dry mass ($\delta_{12,12}$)	0.078(0.027)			0.080
NWG shoot dry mass ($\delta_{14,14}$)	0.083(0.027)			0.085
Alf root dry mass ($\delta_{16,16}$)	0.046(0.014)			0.047
Alf shoot dry mass ($\delta_{18,18}$)	-0.012(0.012)			-0.012

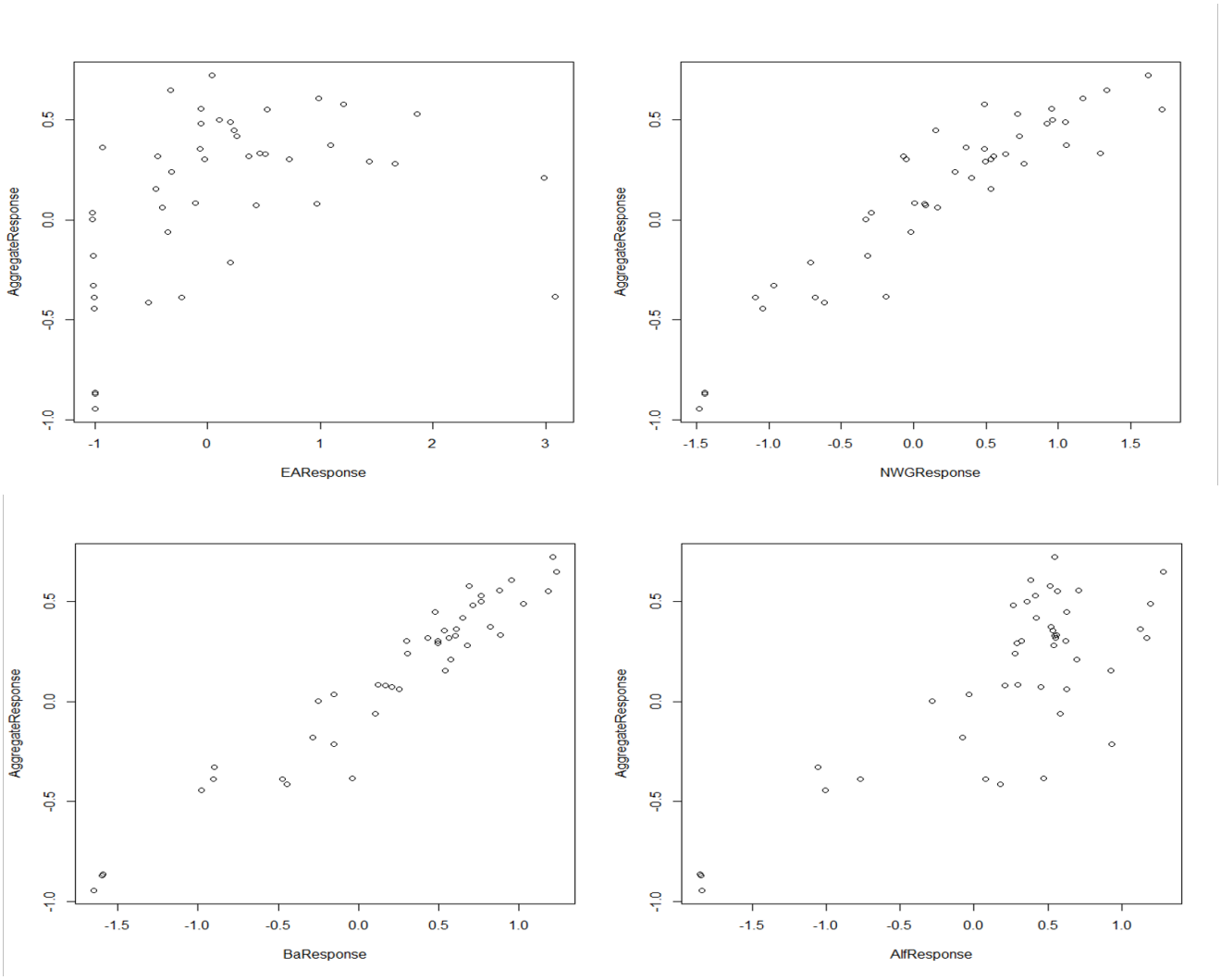


Figure 4.5: Plot of predicted aggregate response variable against the predicted EA response, Ba response, NWG response and Alf response latent variables.
EAresponse - predicted *Eisenia andrei* response; Baresponse - predicted Barley response; NWGresponse - predicted Northern Wheatgrass response; Alfresponse - predicted Alfalfa response for model 3.

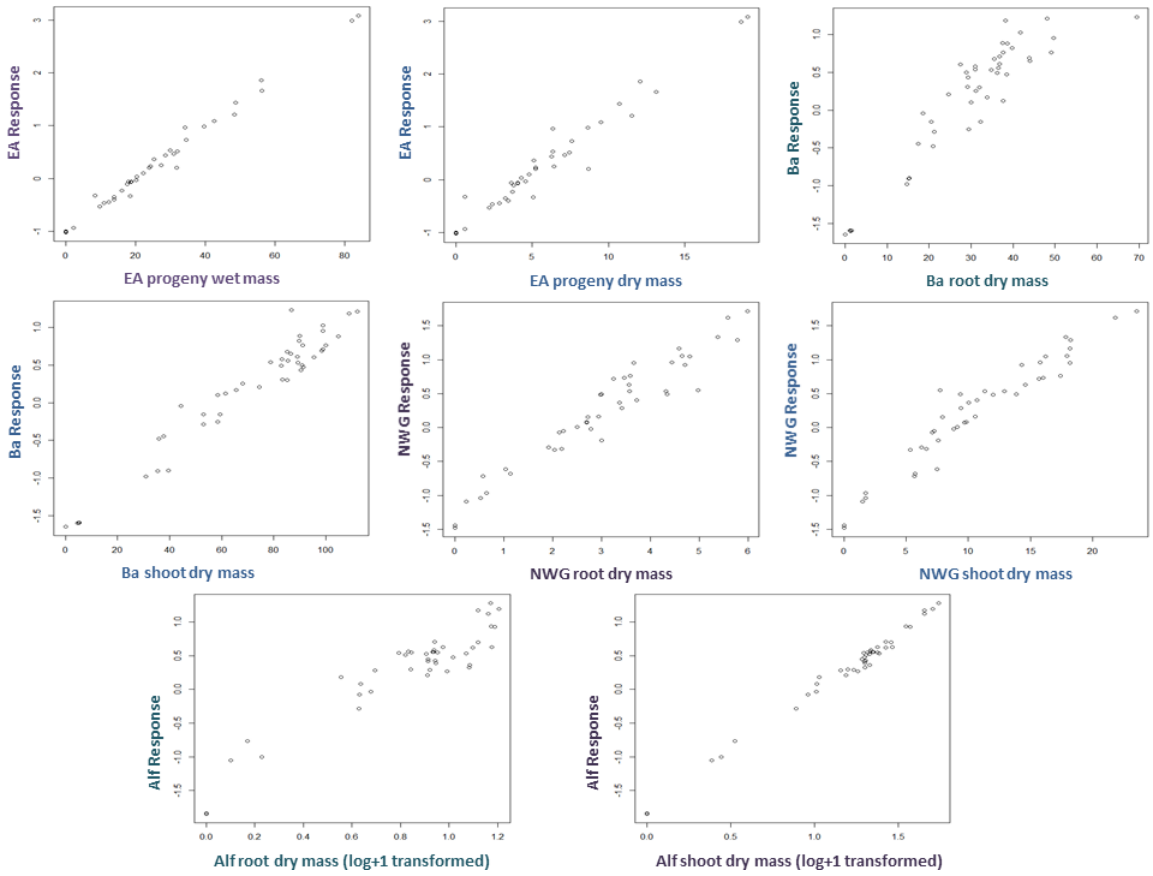


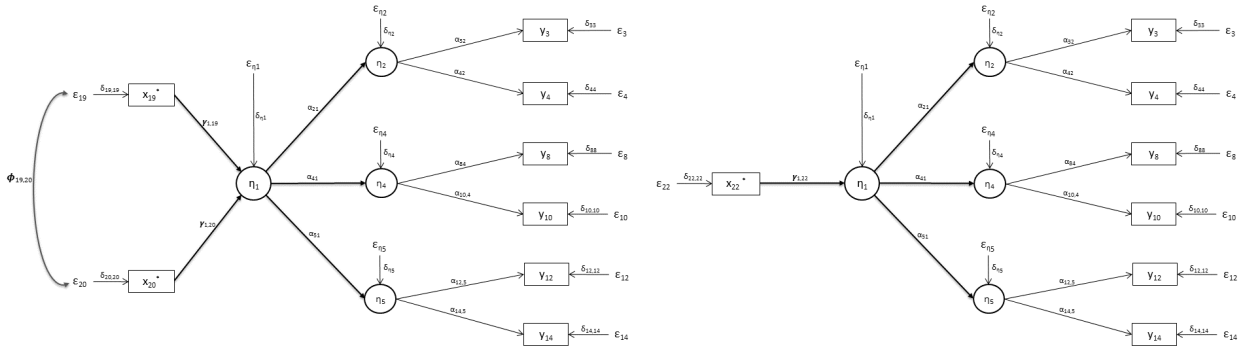
Figure 4.6: Plot of the predicted EA response, Ba response, NWG response and Alf response latent variables against their indicators.
EAresponse - predicted Eisenia Andrei response; Baresponse - predicted Barley response; NWGresponse - predicted Northern Wheatgrass response; Alfresponse - predicted Alfalfa response for model 3.

Table 4.9: Desciption of variables.

Notation	Variable Name	Variable Type	Description
y_3	EA progeny wet mass	Manifest endogenous	The weight of Eisenia andrei progeny before drying
y_4	EA progeny dry mass.	Manifest endogenous	The weight of Eisenia andrei progeny after drying at 60 - 70 degrees C
y_8	Ba root dry mass	Manifest endogenous	The weight of Barley root after drying at 60 - 70 degrees C
y_{10}	Ba shoot dry mass	Manifest endogenous	The weight of Barley shoot after drying at 60 - 70 degrees C
y_{12}	NWG root dry mass	Manifest endogenous	The weight of Northern Wheatgrass root after drying at 60 - 70 degrees C
y_{14}	NWG shoot dry mass	Manifest endogenous	The weight of Northern Wheatgrass shoot after drying at 60 - 70 degrees C
x_{19}	F2	Manifest exogenous	The PHC fraction F2 concentration in the soil
x_{20}	F3	Manifest exogenous	The PHC fraction F3 concentration in the soil
x_{22}	Total PHC	Manifest exogenous	The Total PHC concentration (sum of F2, F3 & F4) in the soil
η_1	Aggregate Response	Latent endogenous	The latent variable representing aggregate species response
η_2	EA Response	Latent endogenous	The latent variable representing Eisenia andrei response
η_4	Ba Response	Latent endogenous	The latent variable representing Barley response
η_5	NWG Response	Latent endogenous	The latent variable representing Northern Wheatgrass response
δ		Estimates	Path coefficients from disturbance to an endogenous variable
α		Estimates	Path coefficients from an endogenous variable to another endogenous variable
γ		Estimates	Path coefficients from an exogenous variable to an endogenous variable
ϵ		Estimates	Disturbance/error for endogenous variables

4.3.3 Relationships between PHC concentrations and aggregate species response

One important use of developing CFA model 3 is the ability to directly model the relationship between the aggregate response of the species and the PHC concentrations in the soil. Two models were specified and compared in this section (Model 4 and 5). The variables are described in Table 4.9. It was assumed in model 4 (Figure 4.7a) that PHC concentration fractions F2, F3 and F4 were individual causes of the aggregate response of the species, and were natural-log + 1 transformed for the purpose of linearization and homoscedasticity. Only F2 (x_{19}) and F3 (x_{20}) were found to be significant. Standardization was carried out on the observed variables before analysis in this section. This involved setting the means and variances of the observed variables to zero and unity respectively. Model 5 (Figure 4.7b) was specified such that the sum of all the PHC fractions (x_{22}) present in the soil was a cause of the aggregate response, and this total PHC variable was natural-log + 1 transformed. The total of the PHC concentration fractions indicated a much stronger relationship between the variables in the model than the individual concentrations of F2 to F4.



(a) model 4

(b) model 5: * is used for variables that have been log+1 transformed

Figure 4.7: Models 4 & 5 modeling the relationship between the aggregate response of the species η_1, and the PHC concentrations in the soil.

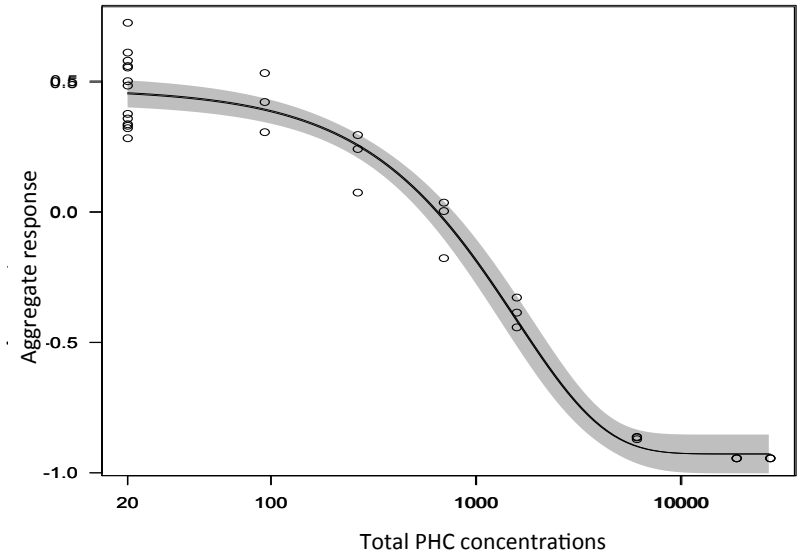
Table 4.10: Model summaries 2 - fit indices for models 4 & 5.
CFI - comparative fit index; TLI- tucker-lewis index; AIC - akaike's information criterion; RMSEA - root mean square error of approximation; SRMR - standardized root mean square residual.

	Model 4 (n=50)	**Model 5 (n=50)**
χ^2	${\chi^2}_{16} = 16.525; p = 0.417$	${\chi^2}_{11} = 11.336; p = 0.416$
CFI	0.999	0.999
TLI	0.999	0.999
AIC	554.597	441.267
RMSEA	0.026; 95% CI 0.000 - 0.134	0.025; 95% CI 0.000 - 0.152
SRMR	0.044	0.020

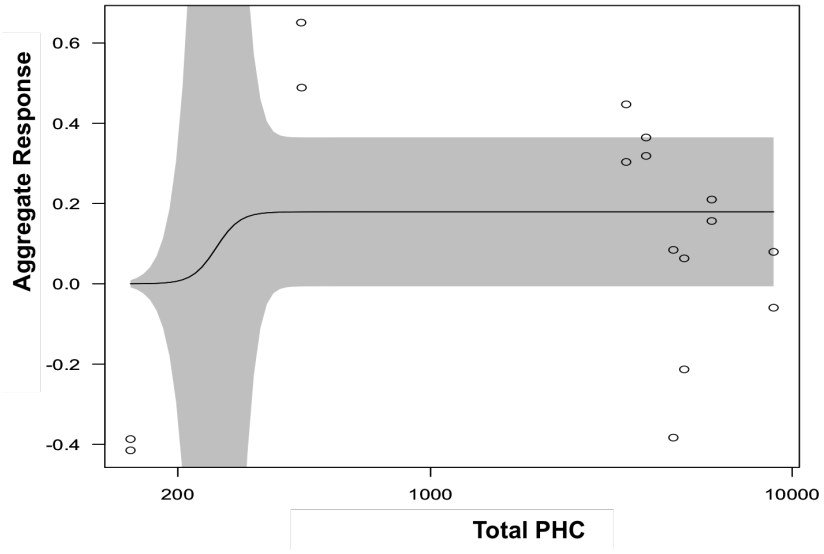
By examination of the results of the analysis (Table 4.10), model 4 and model 5 met all of the criteria for a model with an overall adequate fit (see chapter 3, section 3.5). However, judging from the AIC values, model 5 has an AIC that is 0.79 times the AIC value for model 4. Therefore, even though the fit criteria were satisfied for both models, the better model (Model 5) was chosen based on relative quality. All path coefficients were found to be statistically significant (Table 4.11). Logistic, weibull, and exponential decay models were fit to describe the relationship between the total PHC concentration variable and the aggregate response variable in the individual sites. The three models had AICs of 51.21, 56.81 and 52.89 respectively for site 1; 11.41, 16.48 and 16.57 respectively for site 2. Comparing AICs, the logistic model had an AIC value significantly different (i.e. greater than 2.0) from the AIC value of the Weibull model, but not significantly different from the AIC of the exponential model. The logistic model was however chosen as the best for site 1 and was also chosen as the best model for site 2 based on its AIC value that was significantly low compared to the three models. Figure 4.8 shows the plot of the models for each site including their confidence intervals. Based on the best model for the relationship between the total PHC concentration variable and the aggregate response variable, the IC_{25} values were estimated for individual sites, using the "ED" function in the 'drc' package. The remediation guidelines according to the IC_{25} values were estimated as 452.76 ± 50.38mg/kg for site 1, 234.93 ± 394.78 mg/kg for site 2. Therefore, PHC concentrations above this level will be of concern. These guidelines will be more preferable to use compared to those derived using the current SSD method (see section 4.3.1). This is because the current SSD method uses all end points in its analysis making some species over-represented. This CFA method on the other hand provides a means to know which species end points were providing the same information before guidelines were estimated.

All paths in the final model 5 were significant, and the total PHC concentration fraction was a significant cause of the aggregate species response. The total PHC reduces by 0.44 units for every unit change in the aggregate species response. For all species latent response, each indicator increases by at least 0.90 units for one unit change in standard deviation of its corresponding species response.

Each manifest endogenous variable in model 5 can be represented by the following system



(a) Site 1



(b) Site 2

Figure 4.8: Model 5: scatterplot of the log+1 transformed total PHC concentration variable against the predicted aggregate response variable with bands of confidence intervals.

Table 4.11: Full results for Model 5 including path coefficients and their standard errors (observed variables standardized - col. 2), test of path significance (col. 3), confidence intervals (col. 4), and path coefficients (latent and observed variables standardized - col. 5).

Path	Estimate(Standard error)	P value	Lower CI - Upper CI	Standardized estimates
Latent Variables				
Aggregate response → $EAresponse(\gamma_{21})$	1.00			0.58
Aggregate response → $Baresponse(\gamma_{41})$	1.54(0.33)	<0.001	0.89 - 2.19	0.95
Aggregate response → $NWGresponse(\gamma_{51})$	1.67(0.35)	<0.001	0.99 - 2.36	1.01
EA response → $EAprogenywetmass(\alpha_{32})$	1.00			0.99
EA response → $EAprogenydrymass(\alpha_{42})$	0.99(0.023)	<0.001	0.95 - 1.04	0.99
Ba response → $Barootdrymass(\alpha_{84})$	1.00			0.93
Ba response → $Bashootdrymass(\alpha_{10,4})$	1.04(0.070)	<0.001	0.90 - 1.18	0.96
NWG response → $NWGrootdrymass(\alpha_{12,5})$	1.00			0.94
NWG response → $NWGshootdrymass(\alpha_{14,5})$	1.01(0.064)	<0.001	0.89 - 1.14	0.96
Regressions				
Total PHC → $AggregateResponse(\gamma_{1,22})$	-0.44(0.11)	<0.001	-0.65 - -0.24	-0.78
Variances				
Aggregate response (δ_{η_1})	0.13(0.06)			0.41
EA response (δ_{η_2})	0.66(0.13)			0.67
Ba response (δ_{η_4})	0.085(0.042)			0.099
NWG response (δ_{η_5})	-0.024(0.39)			-0.027
EA progeny wet mass (δ_{33})	-0.002(0.018)			-0.002
EA progeny dry mass (δ_{44})	0.011(0.018)			0.011
Ba root dry mass (δ_{88})	0.12(0.033)			0.12
Ba shoot dry mass ($\delta_{10,10}$)	0.056(0.027)			0.056
NWG root dry mass ($\delta_{12,12}$)	0.094(0.027)			0.094
NWG shoot dry mass ($\delta_{14,14}$)	0.067(0.024)			0.067
Total PHC ($\delta_{22,22}$)	0.98(0.00)			

of linear equations:

$$y_3* = 1 \cdot \eta_2 + \delta_{33}\epsilon_3$$

$$y_4* = \alpha_{42}\eta_2 + \delta_{44}\epsilon_4$$

$$y_8* = 1 \cdot \eta_4 + \delta_{88}\epsilon_8$$

$$y_{10}* = \alpha_{10,4}\eta_4 + \delta_{10,10}\epsilon_{10}$$

$$y_{12} = 1 \cdot \eta_5 + \delta_{12,12}\epsilon_{12}$$

$$y_{14} = \alpha_{14,5}\eta_5 + \delta_{14,14}\epsilon_{14}$$

and each latent endogenous variable can be represented by:

$$\eta_1 = \gamma_{1,22}x_{22}* + \delta_{\eta_1}\epsilon_{\eta_1}$$

$$\eta_2 = 1 \cdot \eta_1 + \delta_{\eta_2}\epsilon_{\eta_2}$$

$$\eta_4 = \alpha_{41}\eta_1 + \delta_{\eta_4}\epsilon_{\eta_4}$$

$$\eta_5 = \alpha_{51}\eta_1 + \delta_{\eta_5}\epsilon_{\eta_5}$$

The vector-matrix notation of the above system of linear equations can be expressed as follows:

$$\begin{bmatrix} \eta \\ y \end{bmatrix} = \mathbb{A} \begin{bmatrix} \eta \\ y \end{bmatrix} + \mathbb{G} \begin{bmatrix} \xi \\ x \end{bmatrix} + \begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} \tag{4.15}$$

where;

$$\mathbb{A}\begin{bmatrix}\eta\\ y\end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & \dots & 0 \\ 1 & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{41} & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{51} & 0 & 0 & 0 & 0 & \dots & 0 \\ 0 & 1 & 0 & 0 & 0 & \dots & 0 \\ 0 & \alpha_{42} & 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & 1 & 0 & 0 & \dots & 0 \\ 0 & 0 & \alpha_{10,4} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{14,5} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{18,6} & 0 & \dots & 0 \end{bmatrix}_{10\times 10} \begin{bmatrix}\eta_1\\ \eta_2\\ \eta_4\\ \eta_5\\ y_3\\ y_4\\ y_8\\ y_{10}\\ y_{12}\\ y_{14}\end{bmatrix}_{10\times 1}, \tag{4.16}$$

$$\eta = \begin{bmatrix}\eta_1\\ \eta_2\\ \eta_4\\ \eta_5\end{bmatrix}_{4\times 1}, y = \begin{bmatrix}y_3*\\ y_4*\\ y_8*\\ y_{10}*\\ y_{12}\\ y_{14}\end{bmatrix}_{6\times 1}, \begin{bmatrix}\eta\\ y\end{bmatrix}_{10\times 1} \tag{4.17}$$

;

$$\mathbb{G}\begin{bmatrix}\xi\\ x\end{bmatrix} = \begin{bmatrix}1\\ \gamma_{1,22}\\ 0\\ 0\\ 0\\ \vdots\\ 0\end{bmatrix}_{10\times 1} \begin{bmatrix}x_{22}*\end{bmatrix}_{1\times 1} \tag{4.18}$$

(the model does not contain latent exogenous variables) ;

$$\begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} = \begin{bmatrix} \Delta_{4\times4} & 0_{4\times6} \\ 0_{6\times4} & \Psi_{6\times6} \end{bmatrix}_{10\times10} \begin{bmatrix} \zeta_{4\times1} \\ \epsilon_{6\times1} \end{bmatrix}_{10\times1} = \tag{4.19}$$

$$\begin{bmatrix} \begin{pmatrix} \delta_{\eta1} & 0 & 0 & 0 \\ 0 & \delta_{\eta2} & 0 & 0 \\ 0 & 0 & \delta_{\eta4} & 0 \\ 0 & 0 & 0 & \delta_{\eta5} \end{pmatrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \\ \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} & \begin{pmatrix} \delta_{33} & 0 & 0 & 0 & 0 & 0 \\ 0 & \delta_{44} & 0 & 0 & 0 & 0 \\ 0 & 0 & \delta_{88} & 0 & 0 & 0 \\ 0 & 0 & 0 & \delta_{10,10} & 0 & 0 \\ 0 & 0 & 0 & 0 & \delta_{12,12} & 0 \\ 0 & 0 & 0 & 0 & 0 & \delta_{14,14} \end{pmatrix} \end{bmatrix}_{10\times10} \begin{bmatrix} \begin{pmatrix} \epsilon_{\eta_1} \\ \epsilon_{\eta_2} \\ \epsilon_{\eta_4} \\ \epsilon_{\eta_5} \end{pmatrix} \\ \begin{pmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \end{pmatrix} \end{bmatrix}_{10\times1} \tag{4.20}$$

,

$$\zeta = \begin{bmatrix} \epsilon_{\eta_1} \\ \epsilon_{\eta_2} \\ \epsilon_{\eta_4} \\ \epsilon_{\eta_5} \end{bmatrix}_{4\times1}, \epsilon = \begin{bmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \end{bmatrix}_{6\times1} \tag{4.21}$$

4.3.4 Relationships between soil characteristics and aggregate species response

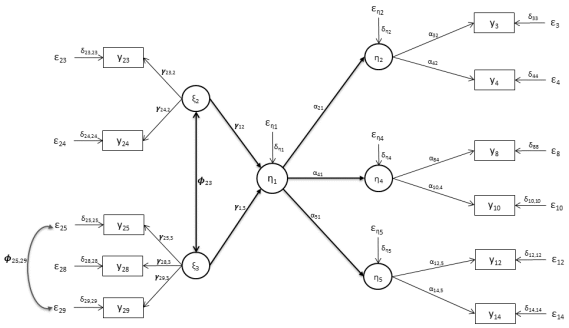
In section 4.3.2, model 3 was found to best model to fit the data and so was used in this section as the form of the preliminary measurement model. Another reason for choosing model 3 is that it included the least number of variables and due to the limit in sample size, a model with fewer variables will lead to more reliable results. Regression methods were applied to the data utilizing the form of model 3 and three different models were used to describe the relationship between soil characteristics and the aggregate species response variable (Figures 4.9a, 4.9b &

Table 4.12: Description of variables.

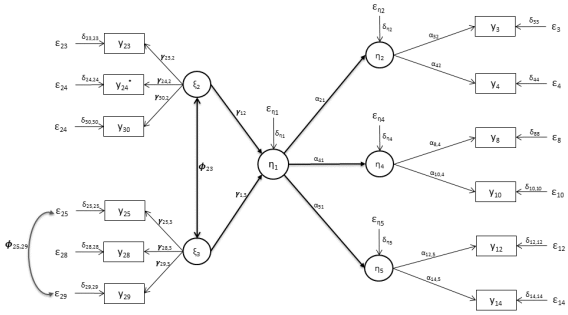
Notation	Variable Name	Variable Type	Description
y_3	EA progeny wet mass	Manifest endogenous	The weight of Eisenia andrei progeny before drying
y_4	EA progeny dry mass.	Manifest endogenous	The weight of Eisenia andrei progeny after drying at 60 - 70 degrees C
y_8	Ba root dry mass	Manifest endogenous	The weight of Barley root after drying at 60 - 70 degrees C
y_{10}	Ba shoot dry mass	Manifest endogenous	The weight of Barley shoot after drying at 60 - 70 degrees C
y_{12}	NWG root dry mass	Manifest endogenous	The weight of Northern Wheatgrass root after drying at 60 - 70 degrees C
y_{14}	NWG shoot dry mass	Manifest endogenous	The weight of Northern Wheatgrass shoot after drying at 60 - 70 degrees C
y_{23}	Silt	Manifest endogenous	The % of Silt in the soil
y_{24}	Clay	Manifest endogenous	The % of Clay in the soil
y_{25}	Total Nitrogen	Manifest endogenous	The % of Total Nitrogen in the soil
y_{28}	pH	Manifest endogenous	The pH level in the Soil
y_{29}	Phosphorous	Manifest endogenous	The Phosphorus content in the Soil
y_{30}	WHC	Manifest endogenous	The Water-Holding Capacity of the soil
η_1	Aggregate Response	Latent endogenous	The latent variable representing aggregate species response
η_2	EA Response	Latent endogenous	The latent variable representing Eisenia andrei response
η_4	Ba Response	Latent endogenous	The latent variable representing Barley response
η_5	NWG Response	Latent endogenous	The latent variable representing Northern Wheatgrass response
ξ_2	Physical properties	Latent exogenous	The latent variable representing the physical properties of the soil
ξ_3	Chemical properties	Latent exogenous	The latent variable representing the chemical properties of the soil
δ		Estimates	Path coefficients from disturbance to an endogenous variable
α		Estimates	Path coefficients from an endogenous variable to another endogenous variable
γ		Estimates	Path coefficients from an exogenous variable to an endogenous variable
ϵ		Estimates	Disturbance/error for endogenous variables

4.9c). All variables used in this section are described in Table 4.12. For all three models in this section, the observed variables were standardized before analysis. Standardization was carried out by setting to zero and unity the means and variances respectively of the observed variables. The models were fit and non-significant paths were removed.

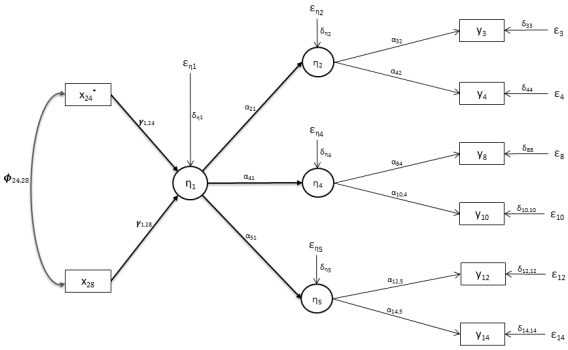
Model 6 (Figure 4.9a) combined the masses by species of *Eisenia andrei*, Barley, and Northern Wheatgrass into latent variables (η_2, η_4, η_5) describing their responses to the PHC contaminant in the soil. These responses were further aggregated into a single latent response variable (η_1). This model incorporated another latent variable (ξ_2) representing the physical properties of the soil indicated by both the silt (y_{23}) and clay (y_{24}) contents of the soil, and another latent variable (ξ_3) representing the chemical properties of the soil indicated by the total amount of nitrogen (y_{25}) in the soil, the pH of the soil (y_{28}), and the total amount of phosphorous in the soil (y_{29}). Significant correlation was identified between the total amount of nitrogen and the total amount of phosphorous in the soil. The physical and chemical latent



(a) model 6



(b) model 7: * is used for variables that have been log+1 transformed



(c) model 8: * is used for variables that have been log+1 transformed

Figure 4.9: Models 6, 7 & 8 describing the relationship between soil characteristics and the aggregate species response variable η_1.

Table 4.13: Model summaries 3 - fit indices for models 6, 7 & 8.
CFI - comparative fit index; TLI- tucker-lewis index; AIC - akaike's information criterion; RMSEA - root mean square error of approximation; SRMR - standardized root mean square residual.

	Model 6 (n=50)	**Model 7 (n=50)**	**Model 8 (n=50)**
χ^2	${\chi^2}_{37} = 61.28; p = 0.007$	${\chi^2}_{46} = 80.019; p<0.001$	${\chi^2}_{16} = 26.20; p = 0.051$
CFI	0.97	0.97	0.98
TLI	0.96	0.95	0.97
AIC	755.003	784.00	534.557
RMSEA	0.12; 95% CI 0.060 - 0.16	0.12; 95% CI 0.075 - 0.17	0.11; 95% CI 0.000 - 0.19
SRMR	0.066	0.066	0.058

variables were specified as predictors of the aggregate species response variables. Model 7 (Figure 4.9b) was a modification of model 6 to include the variable representing the water-holding capacity of the soil (y_{30}) as the third indicator of the physical properties of the soil. In this model, inspection of the variable representing the clay content of the soil indicated a non-linear relationship and the 'clay' variable was linearized by natural-log + 1 transformation. Correlations were found to be statistically significant between the variable representing the water-holding capacity of the soil and the natural-log + 1 transformed clay variable, and also between the total amount of nitrogen in the soil and the phosphorous content of the soil.

The final model (Model 8) that was specified in this section (Figure 4.9c) was the simplest of all three. The model 8 combined the individual mass responses of *Eisenia andrei*, Barley, and Northern wheatgrass into an aggregate response variable predicted directly by the clay content of the soil (x_{24}) and the pH of the soil (x_{28}). The clay variable in model 8 was also natural-log + 1 transformed for linearization purposes.

Table 4.13 gives a brief summary of the fit statistics for each model specified. Based on fit indices, model 8 provided the most adequate fit to the data, with a non-significant chi-square value (χ^2(16, N=50) = 26.20, p = .051), the highest CFI and TLI of 0.98 and 0.97 respectively, the lowest AIC value of 528.56, the lowest RMSEA value (RMSEA = 0.11; 95% CI 0.000 - 0.19) and the lowest SRMR value (0.058).

The transformed clay variable and the pH variable were both significant predictors of the aggregate species response in model 8 (p=0.005 and 0.010 respectively) (Table 4.14). Path coefficients were also highly significant. Standardized estimates show a 0.99 and 0.98

Table 4.14: Full results for Model 8 including path coefficients and their standard errors (observed variables standardized - col. 2), test of path significance (col. 3), confidence intervals (col. 4), and path coefficients (latent and observed variables standardized - col. 5).

Path	Estimate(Standard error)	P value	Lower CI - Upper CI	Standardized estimates
Latent Variables				
Aggregate response → $EAresponse(\gamma_{21})$	1.00			0.58
Aggregate response → $Baresponse(\gamma_{41})$	1.59(0.34)	<0.001	0.92 - 2.26	0.98
Aggregate response → $NWGresponse(\gamma_{51})$	1.64(0.35)	<0.001	0.95 - 2.32	0.98
EA response → $EAprogenywetmass(\alpha_{32})$	1.00			0.99
EA response → $EAprogenydrymass(\alpha_{42})$	0.99(0.023)	<0.001	0.95 - 1.04	0.98
Ba response → $Barootdrymass(\alpha_{84})$	1.00			0.93
Ba response → $Bashootdrymass(\alpha_{10,4})$	1.04(0.070)	<0.001	0.90 - 1.17	0.96
NWG response → $NWGrootdrymass(\alpha_{12,5})$	1.00			0.95
NWG response → $NWGshootdrymass(\alpha_{14,5})$	1.00(0.063)	<0.001	0.87 - 1.12	0.95
Regressions				
Clay → $AggregateResponse(\gamma_{1,24})$	-0.63(0.22)	0.005	-1.06 - -0.19	
pH → $AggregateResponse(\gamma_{1,28})$	-0.55(0.21)	0.010	-0.97 - -0.13	
Variances				
Aggregate response (δ_{η_1})	0.26(0.12)			0.80
EA response (δ_{η_2})	0.66(0.14)			0.67
Ba response (δ_{η_4})	0.033(0.059)			0.038
NWG response (δ_{η_5})	0.031(0.061)			0.034
EA progeny wet mass (δ_{33})	-0.004(0.019)			-0.004
EA progeny dry mass (δ_{44})	0.012(0.019)			0.012
Ba root dry mass (δ_{88})	0.12(0.033)			0.12
Ba shoot dry mass ($\delta_{10,10}$)	0.057(0.026)			0.059
NWG root dry mass ($\delta_{12,12}$)	0.074(0.026)			0.075
NWG shoot dry mass ($\delta_{14,14}$)	0.088(0.028)			0.090
Clay	0.98(0.00)			
pH	0.98(0.00)			
Covariance between Clay and pH ($\delta_{24,28}$)	-0.89(0.00)			

unit change in the wet mass and dry mass respectively of *Eisenia andrei* per unit standard deviation in the total *Eisenia andrei* response. For one unit change in standard deviation of Barley response, there is a 0.93 unit change in the root dry mass of Barley and a 0.96 unit change in the shoot dry mass of Barley, while for one unit change in standard deviation of Northern Wheatgrass response, there is a 0.95 unit change in the root dry mass of Northern Wheatgrass and a 0.96 unit change in the shoot dry mass of Northern Wheatgrass. A strong negative correlation was found between the transformed clay variable and the pH variable - one unit change in clay results in a 0.89 decrease in one unit pH.

Using the LISREL system of linear equations, the observed endogenous variables in model

6 can be represented by:

$$y_3 = \alpha_{32}\eta_2 + \delta_{33}\epsilon_3$$

$$y_4 = \alpha_{42}\eta_2 + \delta_{44}\epsilon_4$$

$$y_8 = \alpha_{84}\eta_4 + \delta_{88}\epsilon_8$$

$$y_{10} = \alpha_{10,4}\eta_4 + \delta_{10,10}\epsilon_{10}$$

$$y_{12} = \alpha_{12,5}\eta_5 + \delta_{12,12}\epsilon_{12}$$

$$y_{14} = \alpha_{14,5}\eta_5 + \delta_{14,14}\epsilon_{14}$$

and the latent endogenous variables can be represented by:

$$\eta_1 = \gamma_{1,24}x_{24}* + \gamma_{1,28}x_{28} + \delta_{\eta_1}\epsilon_{\eta_1}$$

$$\eta_2 = \alpha_{21}\eta_1 + \delta_{\eta_2}\epsilon_{\eta_2}$$

$$\eta_4 = \alpha_{41}\eta_1 + \delta_{\eta_4}\epsilon_{\eta_4}$$

$$\eta_5 = \alpha_{51}\eta_1 + \delta_{\eta_5}\epsilon_{\eta_5}$$

The vector-matrix notation can be expressed as follows:

$$\begin{bmatrix} \eta \\ y \end{bmatrix} = \mathbb{A} \begin{bmatrix} \eta \\ y \end{bmatrix} + \mathbb{G} \begin{bmatrix} \xi \\ x \end{bmatrix} + \begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} \tag{4.22}$$

where;

$$\mathbb{A}\begin{bmatrix}\eta\\ y\end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{31} & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{41} & 0 & 0 & 0 & 0 & \dots & 0 \\ \alpha_{51} & 0 & 0 & 0 & 0 & \dots & 0 \\ 0 & \alpha_{32} & 0 & 0 & 0 & \dots & 0 \\ 0 & \alpha_{42} & 0 & 0 & 0 & \dots & 0 \\ 0 & 0 & \alpha_{84} & 0 & 0 & \dots & 0 \\ 0 & 0 & \alpha_{10,4} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{12,5} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{14,5} & 0 & 0 & \dots & 0 \\ 0 & 0 & 0 & \alpha_{18,6} & 0 & \dots & 0 \end{bmatrix}_{10\times 10} \begin{bmatrix}\eta_1\\ \eta_2\\ \eta_4\\ \eta_5\\ y_3\\ y_4\\ y_8\\ y_{10}\\ y_{12}\\ y_{14}\end{bmatrix}_{10\times 1} \tag{4.23}$$

,

$$\eta = \begin{bmatrix}\eta_1\\ \eta_2\\ \eta_4\\ \eta_5\end{bmatrix}_{4\times 1}, y = \begin{bmatrix}y_3\\ y_4\\ y_8\\ y_{10}\\ y_{12}\\ y_{14}\end{bmatrix}_{6\times 1}, \begin{bmatrix}\eta\\ y\end{bmatrix}_{10\times 1} \tag{4.24}$$

;

$$\mathbb{G}\begin{bmatrix}\xi\\ x\end{bmatrix} = \begin{bmatrix} 1 & \\ \gamma_{1,24} & \gamma_{1,28} \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ \vdots & \vdots \\ 0 & 0 \end{bmatrix}_{10\times 2} \begin{bmatrix} x_{24}* & x_{28}\end{bmatrix}_{2\times 1} \tag{4.25}$$

(the matrix $\begin{bmatrix}\xi\\ x\end{bmatrix}$ does not contain ξ because there are no latent exogenous variables in the model) ;

$$\begin{bmatrix} \Delta & 0 \\ 0 & \Psi \end{bmatrix} \begin{bmatrix} \zeta \\ \epsilon \end{bmatrix} = \begin{bmatrix} \Delta_{4\times4} & 0_{4\times6} \\ 0_{6\times4} & \Psi_{6\times6} \end{bmatrix}_{10\times10} \begin{bmatrix} \zeta_{4\times1} \\ \epsilon_{6\times1} \end{bmatrix}_{10\times1} =$$

$$\begin{bmatrix} \begin{pmatrix} \delta_{\eta1} & 0 & 0 & 0 \\ 0 & \delta_{\eta2} & 0 & 0 \\ 0 & 0 & \delta_{\eta4} & 0 \\ 0 & 0 & 0 & \delta_{\eta5} \end{pmatrix} & \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \\ \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} & \begin{pmatrix} \delta_{33} & 0 & 0 & 0 & 0 & 0 \\ 0 & \delta_{44} & 0 & 0 & 0 & 0 \\ 0 & 0 & \delta_{88} & 0 & 0 & 0 \\ 0 & 0 & 0 & \delta_{10,10} & 0 & 0 \\ 0 & 0 & 0 & 0 & \delta_{12,12} & 0 \\ 0 & 0 & 0 & 0 & 0 & \delta_{14,14} \end{pmatrix} \end{bmatrix}_{10\times10} \begin{bmatrix} \begin{pmatrix} \epsilon_{\eta_1} \\ \epsilon_{\eta_2} \\ \epsilon_{\eta_4} \\ \epsilon_{\eta_5} \end{pmatrix} \\ \begin{pmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \end{pmatrix} \end{bmatrix}_{10\times1},$$

$$\zeta = \begin{bmatrix} \epsilon_{\eta_1} \\ \epsilon_{\eta_2} \\ \epsilon_{\eta_4} \\ \epsilon_{\eta_5} \end{bmatrix}_{4\times1}, \epsilon = \begin{bmatrix} \epsilon_3 \\ \epsilon_4 \\ \epsilon_8 \\ \epsilon_{10} \\ \epsilon_{12} \\ \epsilon_{14} \end{bmatrix}_{6\times1} \tag{4.26}$$

4.3.5 Conclusion

This chapter has described methods for analyzing toxicological responses. Most important is the use of CFA to aggregate responses of species to PHC contamination in soil and building models to predict these responses across a range of PHC concentrations. The CFA method is useful for identifying the species endpoints that respond differently compared to other endpoints by examining path significance. This is valuable because it ensures that all the endpoints in the model are collectively functional in the study.

This analysis has also shown the utility of SEM to directly examine the relationship between the aggregate responses of species and environmental variables.

Chapter 5

Discussion

The issue of soil contamination is one of great concern as there are about 4,700 soils contaminated with petroleum hydrocarbons (PHCs). An extensive literature review was conducted to explain the fact that there is presently a challenge in developing remediation targets for the many soil types that have been contaminated by PHCs. The big question is "how do we know to which level of PHC concentration to clean these sites", enough to mitigate ecological damage. A major challenge in deriving these levels comes mostly from the fact that there are variations in soil and environmental conditions from site to site. This project demonstrated that we could predict toxicological effects in a given soil when we have variables that give information about the contaminant concentrations and either the species endpoints or the soil characteristics.

The primary objectives of the study were to:

- To develop site-specific remedial objectives for soils contaminated with petroleum hydrocarbons based on readily measured environmental variables and soil characteristics.
- To assess toxicological responses by modeling the relationships among contaminant concentrations and either species endpoints or soil characteristics.
- To summarize the aggregate response of species using a single-valued estimate, IC_{25}.

As a standard, estimated IC_{25} values are used as a standard for PHC to assess the toxicity of contaminated soils. The methods used to obtain an IC_{25} value is therefore as important as the value itself. The remediation guidelines for a site (SSROs) are based on a policy decision to use the 25th percentile of the distribution of IC_{25}s generated for different species and endpoints (i.e., Species sensitivity distribution, SSD) as a protection level for

agricultural/residential land uses. First, non-linear procedures are used for quantifying the relationships between total PHC concentrations and individual species endpoints to estimate IC_{25} values for each endpoint. Next, the estimated IC_{25}s are combined to develop a species sensitivity distribution (SSD). A final IC_{25} is then computed from this cumulative distribution to provide an estimate for remediation objectives. The present SSD method was applied to data from the two sites in this study and remediation guidelines according to the final IC_{25}s were estimated as 258.3mg/kg for site 1 and 107.3mg/kg for site 2.

The SSD method is known for clarity, simplicity in analysis, and is used for decision making by risk assessors (Angell *et al.*, 2012). However, it has some flaws. It's reliability has not been put to test in comparison to other methods; it requires relatively large datasets and as seen in section 4.3.1 some of the species were overrepresented (Whitacre, 2010). The SSD method does not provide a way to determine what species endpoints give similar information or are as important as other endpoints to know which endpoints to include or exclude from the model. The use of this method can also questioned because it derives a single IC_{25} estimate based on other IC_{25} estimates. This study presents the utility of structural equation modeling (SEM) to solve some of the problems posed by the SSD method.

Confirmatory factor analysis procedures were applied to the dataset to estimate the latent variables. Log + 1 transformation was carried out on the observed variables in situations where assumptions associated with the regression model (linearity, normality, homoscedasticity) were not satisfied. From the CFA, endpoints that had nonsignificant path loadings on a latent variable were identified. This procedure can be utilized by toxicologists to determine whether all the endpoints are providing the same information about the latent variable. The final CFA model (Model 3 - Figure 4.4c) estimated an aggregate species response variable based on the individual responses of each species each indicated by their masses. Significant path loadings in the CFA model (Table 4.8) showed that the species masses were responding in the same way and also providing similar information about the latent variables that they were indicators of. Model 5 (Figure 4.7b) showed that the total PHC concentration present in the soil was a significant cause of the aggregate response of the soil (Table 4.11). The total PHC concentrations indicated a stronger relationship with the aggregate species response than the individual concentrations of F2 to F4. Model 8 (Figure 4.9c) described the causal

effect of the pH and clay content of the soil on the aggregate response of the species, also showing that the two soil characteristics were significantly correlated (Table 4.14). Studies have also shown that increased clay content causes a decrease in the bioavailability of hydrocarbons resulting from the fact that clay content can absorb hydrocarbons (El-Tarabily, 2002; Eibes et al., 2006).

Individual site analysis showed that for site 1, a logistic model best described the relationship between the total PHC concentrations and the aggregate species response, while for site 2, a logistic model gave the best explanation for this relationship. IC_{25} values for each model were estimated for individual sites, and these values will be used as remediation guidelines. These values were 452.76 $\pm$ 50.38mg/kg for site 1 and 234.93 $\pm$ 394.78 mg/kg for site 2. Therefore, for site 1, or any of the 4700 contaminated sites in Canada with similar soil characteristics, any PHC exposures above the range of 452.76 $\pm$ 50.38mg/kg will be of concern and can result in immediate or long term hazard to the environment. Similarly, for site 2, or any other sites with similar soil properties, concentrations above the range of 234.93 $\pm$ 394.78 mg/kg will be considered hazardous. One recommendation, therefore, is to partition all the contaminated sites that we have in Canada into homogenous groups based on their soil properties and analyze samples from each group. Stratification can also be carried out according to soil types to determine how toxicological effects can differ across soil types.

The long-term goal of this study is to help toxicologists, regulators, assessors, and managers come up with more reliable site-specific remediation objectives (SSROs) on a site-by-site basis. Ideally, a toxicologist would be able to measure species endpoint and soil/environment variables, and using the methods that were developed in this study; they can determine toxicity values without performing extensive procedures (see chapter 2, section 2.1). As shown in this project, confirmatory factor analysis was successfully used to combine different responses of species to petroleum hydrocarbons by taking into account measured species endpoints variables. This combined response was further incorporated into standard non-linear procedures to estimate IC_{25} values for remediation.

5.1 Strength, Limitations and Further Scope

Strengths of the study

This is another of the few studies targeted toward providing an alternative towards current methods used in toxicology to derive soil quality guidelines. The study has shown the possibility to model directly the relationship between the predicted aggregate responses and PHC contaminant concentrations using the latent variable modeling technique known as SEM. Using confirmatory factor analysis, toxicologists can determine what endpoints are providing similar information before going ahead to estimate IC_{25} values using standard procedures. These important endpoints can be determined by examining their path loadings on the latent variables they are indicators of. These endpoints may vary from study to study since an insignificant path loading may only be exclusive to a particular study and may be significant in another study. This is one advantage that the SEM methodology has over the current SSD method, which considers all endpoints as similarly important.

Limitations of the study

One limitation was the lack of consistency in the availability of species across the different sites. Endpoint data for barley, northern wheatgrass, perennial ryegrass, alfalfa, red clover, earthworm, collembolan, *Folsomi candida* were collected from different samples across the sites; however, only data for earthworm, *Folsomi candida*, barley, Northern Wheatgrass, and alfalfa were complete for all study sites and used for analysis. This issue arises from the fact that some species can only be observed at specific sites because of differences in environmental properties and species adaptability across sites.

The sample size was a total of 50, which is small relative to the sample size requirement for SEM. The sample size requirement to carry out an SEM analysis ($N \geqslant 8K$, where K is the number of observed variables in the model) is quite restrictive because larger datasets may not be feasible to obtain in toxicology. Ecologists have discussed this issue extensively and, therefore, advise that it is better to analyze with smaller datasets than not at all (Grace, 2006). Two sites used in this study differed greatly in sample size. Site 1 had a sample size of 34 which was more than twice the sample size for site 2, which had a sample size of 16.

This made it difficult to perform individual site analysis on site 2. With more data, there is more chance of the sample being a good representative of the whole site.

Further scope Further analysis should be conducted using soils for other sites and endpoint variables from other species. It will also be useful to incorporate soils from different sites to provide some more variability in soil properties. This will help to determine how responses can differ based on different types of soils.

The methods in this study can be used to develop remediation guidelines from contaminated soils; however, the models should be validated by assessing the accuracy of the predictions by verifying the predictions with toxicological assessments.

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Appendix A

Dataset

		Plant Species														
		Ba (Barley)					NWG (Northern Wheatgrass)					Alf (Alfalfa)				
	Study	Emergence	Shoot length	Root length	Shoot dry mass	Root dry mass	Emergence	Shoot length	Root length	Shoot dry mass	Root dry mass	Emergence	Shoot length	Root length	Shoot dry mass	Root dry mass
1	1	100	219.8	200	104.72	38.62	100	161	106.2	18.18	3.66	100	65	170.8	25.75	7.73
2	1	100	204.2	189.2	109	38.14	100	163.8	139.4	23.54	5.98	100	62	165	20.57	5.8
3	1	100	206.8	171.4	89.34	34.76	80	124.5	83	13.88	3	100	60.7	164.1	23.37	10.76
4	1	100	222.4	206.8	111.98	48.12	100	156.8	118	21.82	5.58	100	64.7	174.3	22.86	7.98
5	1	100	215	160.4	99.98	37.62	100	148.4	106.8	15.8	4.44	70	54.3	142.6	20.41	11.19
6	1	100	219.4	170.6	98.84	49.62	100	150	108	18.2	4.58	100	55.4	146.7	19.09	7.83
7	1	100	205.4	185.4	85.38	36.56	80	140.5	109	7.75	4.98	100	58.9	151.9	21.24	7.55
8	1	100	214.3	177.8	98.75	36.85	100	145.8	105.4	14.28	4.7	100	48.8	161.2	17.23	8.79
9	1	100	210.6	215.6	85.22	35.54	100	155.4	96.2	17.4	3.58	100	59.5	136.3	18.68	5.21
10	1	100	212.8	179.8	90.06	37.52	100	148.6	116.8	18.24	5.78	100	59.6	141.2	21.48	7.62
11	1	100	217	165	98.44	43.92	60	135.3	85	12	2.97	100	63.4	190.3	19.46	5.6
12	1	100	217	167.4	95.34	27.44	100	140.6	100.6	14.6	3.56	90	58.3	142.2	19.71	5.98
13	1	100	195.6	179.4	89.82	39.78	100	141	100.2	17.94	4.64	100	59.6	148	20.41	7.07
14	1	100	209	155.6	90.76	28.86	80	137	97	12.9	3.57	100	51.2	166.9	19.02	11.06
15	1	100	205.8	174.4	86.58	44.04	100	154.6	84.4	16.04	3.46	100	55.9	160.4	19.17	7.75
16	1	100	208.8	182.2	91.2	49.04	100	151.8	84.8	15.68	3.24	90	53.4	161.4	18.97	7.2
17	1	100	208.8	155.8	83.26	29.18	100	135	80	9.42	3.42	80	39.4	95.3	13.31	3.96
18	1	100	191.8	154.4	74.36	24.66	100	139	75.2	9.84	2.7	90	49.9	121.1	18.24	7.17
19	1	100	207	149.6	82.88	36.24	100	112.2	77.8	9.36	4.34	100	48	110.2	16.24	7.34
20	1	100	185.6	107.2	52.94	21.26	80	118.3	62.3	6.62	2.18	100	36.3	69.3	8.19	3.28
21	1	100	193	128.4	58.38	29.42	100	93.2	57.6	5.32	2.04	80	35.9	62.1	6.8	3.26
22	1	100	195.8	123	59.38	32.24	100	97.4	62.8	6.26	1.92	100	36	70.6	9.26	3.77
23	1	80	144	43.5	39.45	15.25	80	49.8	21	1.75	0.65	50	13.2	13	1.44	0.26
24	1	100	148.4	54.8	35.36	15.16	60	49	16.3	1.5	0.23	60	13.8	20.3	2.34	0.48
25	1	100	125	49.4	30.74	14.76	80	46.3	21.5	1.75	0.53	70	12.6	20.7	1.77	0.69
26	1	100	23.8	5	5.1	1.56	0	0	0	0	0	0	0	0	0	0
27	1	80	27	4.7	5.35	1.1	0	0	0	0	0	0	0	0	0	0
28	1	80	29.5	4.5	4.62	1.3	0	0	0	0	0	0	0	0	0	0
29	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	2	100	136.2	165.2	37.58	17.4	100	100.8	54.2	7.5	1.04	90	39.1	107.1	9.69	2.59
36	2	80	135.5	179.8	35.8	20.93	60	113	49.7	5.7	1.13	60	35.3	167.5	9.38	3.32
37	2	80	287.8	288.5	98.85	41.8	100	166.6	160.4	16.22	4.8	40	118.5	215.5	49.73	15
38	2	100	268.6	306.2	86.84	69.4	100	203	215.2	17.84	5.38	80	109	234	54	13.81
39	2	100	280.6	264.4	90.48	29.3	60	151.7	147.3	7.1	2.13	80	123.3	217.8	44.43	12.15
40	2	100	278.2	268.2	89.02	36.82	80	172	158.5	10.03	3.37	90	128.8	263	44.3	13.5
41	2	100	232.2	253.2	53.02	20.46	80	122.5	50	5.65	0.58	60	102.8	221.7	34.22	13.9
42	2	80	265.5	283	68.15	31.15	100	172.8	129.2	10.58	2.94	50	84.8	208.8	22.74	8.42
43	2	100	282	255	91.4	38.48	80	148	137.5	7.9	2.72	100	90.6	181.3	28.2	14.03
44	2	100	270.2	239.8	85.08	31.92	80	134	125	7.23	2.22	80	99.4	156	25.78	11.49
45	2	100	239	231.8	61.52	37.7	60	158.7	106	9.13	2.5	70	53	200.4	14.87	5.96
46	2	100	214.4	166.8	44.4	18.56	80	139.5	107	7.58	3	70	61	163.3	18.89	9.37
47	2	100	284.6	238.4	78.78	31.04	80	160.5	192.8	11.33	4.32	100	112.9	267.6	36.3	14.46
48	2	60	297	241	83.17	30.97	100	173.2	170.6	10.7	3.72	100	85.2	223.7	27.81	12.19
49	2	100	224.8	257.8	65.56	33.76	80	154.8	80	9.72	2.7	50	60.2	171.4	14.42	7.14
50	2	100	230.8	244.4	58.36	30.06	100	157.8	114.2	8.82	2.78	60	87.2	153.3	20.8	7.65

		Invertebrate Species					
		EA (Eisenia andrei - Earthworm)				FC (Folsomia candida)	
	Study	Survival	Progeny	Progeny wet mass	Progeny dry mass	Survival	Progeny
1	1	100	8	18.84	4.04	8	507
2	1	100	16	29.98	6.36	10	569
3	1	100	10	18.73	4.06	6	422
4	1	100	12	20.38	4.28	7	539
5	1	100	15	22.17	4.8	10	389
6	1	100	25	39.66	8.66	9	451
7	1	100	40	25.37	5.09	5	423
8	1	100	18	17.91	3.63	10	428
9	1	100	23	56.35	13.1	8	425
10	1	100	25	30.92	7.11	7	558
11	1	100	18	48.35	11.54	10	514
12	1	100	19	32.06	7.42	10	521
13	1	100	14	42.54	9.49	8	415
14	1	100	34	34.62	7.59	7	294
15	1	100	23	27.3	6.43	9	391
16	1	100	14	56.1	12.08	10	603
17	1	100	3	8.43	0.6	7	409
18	1	100	11	28.65	6.26	8	468
19	1	100	7	48.74	10.71	6	436
20	1	100	0	0	0	8	358
21	1	100	0	0	0	8	473
22	1	100	0	0	0	9	280
23	1	0	0	0	0	0	0
24	1	0	0	0	0	7	0
25	1	100	0	0	0	0	0
26	1	0	0	0	0	0	0
27	1	0	0	0	0	0	0
28	1	0	0	0	0	0	0
29	1	0	0	0	0	0	0
30	1	0	0	0	0	0	0
31	1	0	0	0	0	0	0
32	1	0	0	0	0	0	0
33	1	0	0	0	0	0	0
34	1	0	0	0	0	0	0
35	2	100	22	9.77	2.2	90	870
36	2	100	18	16.14	3.72	40	881
37	2	100	81	31.8	8.7	80	1358
38	2	100	99	18.41	5.07	60	1284
39	2	100	45	12.28	2.86	100	541
40	2	100	14	2.28	0.6	90	831
41	2	100	59	23.92	5.22	90	1485
42	2	100	31	13.84	3.41	80	629
43	2	100	43	24.34	5.2	80	68
44	2	100	74	20.23	4.56	70	448
45	2	100	63	17.68	3.79	70	324
46	2	100	16	83.89	19.09	90	495
47	2	100	66	10.96	2.37	90	1868
48	2	100	8	82.14	18.69	100	442
49	2	100	30	34.24	6.35	60	1111
50	2	100	62	13.94	3.23	90	1047

											Soil Properties										
	Study	Soil Moisture	pH	Conductivity	WHC	Total Nitrogen	Total Carbon	InCarbon	OrgCarbon	Phosphorous	Organic Matter	Gravel	Sand	VeryFineSand	FineSand	MediumSand	CoarseSand	VCourseSand	Silt	Clay	Texture
1	1	42	5.22	1270	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
2	1	42	5.22	1270	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
3	1	42	5.22	1270	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
4	1	42	5.22	1270	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
5	1	34	5.21	1310	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
6	1	34	5.21	1310	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
7	1	34	5.21	1310	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
8	1	34	5.2	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
9	1	34	5.2	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
10	1	34	5.2	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
11	1	33	5.21	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
12	1	33	5.21	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
13	1	33	5.21	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
14	1	32	5.2	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
15	1	32	5.2	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
16	1	32	5.2	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
17	1	32	5.17	1450	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
18	1	32	5.17	1450	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
19	1	32	5.17	1450	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
20	1	32	5.16	1440	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
21	1	32	5.16	1440	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
22	1	32	5.16	1440	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
23	1	32	5.71	1220	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
24	1	32	5.71	1220	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
25	1	32	5.71	1220	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
26	1	32	5.57	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
27	1	32	5.57	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
28	1	32	5.57	1300	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
29	1	34	5.47	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
30	1	34	5.47	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
31	1	34	5.47	1360	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
32	1	42	5.53	773	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
33	1	42	5.53	773	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
34	1	42	5.53	773	68	0.52	5.24	0	5.24	22	9.1	0.15	30.1	12.95	11.95	4.4	0.85	0	42.45	27.45	
35	2	42	6.3	39	40	0.02	0.89		0.87	7	6		56						20	24	Fine sandy loam
36	2	42	6.45	39	26.6	0.08	0.89		0.89	3.86	1.6		37						41	22	Fine sandy loam
37	2	57	7.13	211	41	0.11	2.23		1.68	52	5.2		56						28	16	Fine sandy loam
38	2	57	7.55	211	12.5	0.15	2.23		2.23	31.5	3.8		53						35	12	Fine sandy loam
39	2	58	7.18	541	41	0.29	5.26		3.29	110	7.6		67						13	20	Sandy Loam
40	2	58	7.24	533	14.6	0.21	5.26		5.26	99.4	7.7		50.3						34	15	Sandy Loam
41	2	64	7.29	438	37	0.17	4.52		3.57	100	7.8		62						17	21	Loam
42	2	64	7.28	440	13.1	0.15	4.52		4.52	119	7.6		50						34	16	Loam
43	2	61	7.5	574	40	0.21	3.96		3.53	48	6.9		60						30	10	Sandy Loam
44	2	61	7.48	553	14.1	0.17	3.96		3.96	49.4	7.4		50						34	13	Sandy Loam
45	2	50	7.66	326	38	0.13	3.86		3.39	46	6.9		56						24	20	Loam
46	2	50	7.58	314	14.2	0.14	3.86		3.86	48.6	7		55						33	16	Loam
47	2	55	7.6	371	42	0.14	4.88		4.85	36	7.3		66						18	16	Sandy Loam
48	2	55	7.63	373	16.1	0.16	4.88		4.88	41.7	9.1		51						32	14	Sandy Loam
49	2	66	7.76	257	36	0.1	6.41		5.01	26	8		63						19	18	Loam
50	2	66	7.67	259	14	0.14	6.41		6.41	39.4	8.5		53						34	16	Loam

		PHC Concentrations							
	Study	F2	logF2	F3	logF3	F4	logF4	TotalPHC	logTotalPHC
1	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
2	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
3	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
4	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
5	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
6	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
7	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
8	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
9	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
10	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
11	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
12	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
13	1	5	0.77815125	5	0.77815125	10	1.04139269	20	1.3222193
14	1	78	1.89762709	5	0.77815125	10	1.04139269	93	1.97312785
15	1	78	1.89762709	5	0.77815125	10	1.04139269	93	1.97312785
16	1	78	1.89762709	5	0.77815125	10	1.04139269	93	1.97312785
17	1	249	2.39794001	5	0.77815125	10	1.04139269	264	2.42324587
18	1	249	2.39794001	5	0.77815125	10	1.04139269	264	2.42324587
19	1	249	2.39794001	5	0.77815125	10	1.04139269	264	2.42324587
20	1	678.5	2.83218946	5	0.77815125	10	1.04139269	693.5	2.84167225
21	1	678.5	2.83218946	5	0.77815125	10	1.04139269	693.5	2.84167225
22	1	678.5	2.83218946	5	0.77815125	10	1.04139269	693.5	2.84167225
23	1	1556.67	3.19247546	5	0.77815125	10	1.04139269	1571.67	3.1966376
24	1	1556.67	3.19247546	5	0.77815125	10	1.04139269	1571.67	3.1966376
25	1	1556.67	3.19247546	5	0.77815125	10	1.04139269	1571.67	3.1966376
26	1	6006.67	3.77870607	29.33	1.48187241	10	1.04139269	6046	3.78153997
27	1	6006.67	3.77870607	29.33	1.48187241	10	1.04139269	6046	3.78153997
28	1	6006.67	3.77870607	29.33	1.48187241	10	1.04139269	6046	3.78153997
29	1	18233.33	4.26088981	271.67	2.43563736	10	1.04139269	18515	4.26754717
30	1	18233.33	4.26088981	271.67	2.43563736	10	1.04139269	18515	4.26754717
31	1	18233.33	4.26088981	271.67	2.43563736	10	1.04139269	18515	4.26754717
32	1	26800	4.428151	193.67	2.28929903	10	1.04139269	27003.67	4.43143888
33	1	26800	4.428151	193.67	2.28929903	10	1.04139269	27003.67	4.43143888
34	1	26800	4.428151	193.67	2.28929903	10	1.04139269	27003.67	4.43143888
35	2	5	0.77815125	65	1.81954394	78	1.89762709	148	2.17318627
36	2	5	0.77815125	65	1.81954394	78	1.89762709	148	2.17318627
37	2	15	1.20411998	196	2.29446623	229	2.36172784	440	2.64443859
38	2	13.81	1.17055506	196	2.29446623	229	2.36172784	438.81	2.6432651
39	2	5	0.77815125	1850	3.26740642	2090	3.32035403	3945	3.59615708
40	2	5	0.77815125	1850	3.26740642	2090	3.32035403	3945	3.59615708
41	2	39	1.60205999	2510	3.39984671	2480	3.39462676	5029	3.70156799
42	2	39	1.60205999	2510	3.39984671	2480	3.39462676	5029	3.70156799
43	2	5	0.77815125	1610	3.20709554	1860	3.26974637	3475	3.54107977
44	2	5	0.77815125	1610	3.20709554	1860	3.26974637	3475	3.54107977
45	2	13	1.14612804	2250	3.3523755	2430	3.38578496	4693	3.67154309
46	2	13	1.14612804	2250	3.3523755	2430	3.38578496	4693	3.67154309
47	2	11	1.07918125	2630	3.42012085	3350	3.52517443	5991	3.77757181
48	2	11	1.07918125	2630	3.42012085	3350	3.52517443	5991	3.77757181
49	2	53	1.73239376	4170	3.62024019	4660	3.6684791	8883	3.94860855
50	2	53	1.73239376	4170	3.62024019	4660	3.6684791	8883	3.94860855

Appendix B

Software Implementation of data analysis

```
    library(lavaan)
library(sem)
library(semPlot)
library(semTools)
library(fitdistrplus)
```

Model 1

```
model1 <- 'Agg.resp =~ EA_progeny + EA$_$progeny_wet_mass + EA_progeny_dry_mass
+ FC_survival + FC_progeny + Ba_root_dry_mass + Ba_shoot_dry_mass + Ba_root_length
+ Ba_shoot_length + NWG_shoot_dry_mass + NWG_root_dry_mass + NWG_shoot_length +
NWG_root_length + Alf_shoot_dry_mass + Alf_root_dry_mass + Alf_shoot_length +
Alf_root_length
EA_progeny ~ ~ EA_progeny_wet_mass
EA_progeny_wet_mass ~ ~ EA_progeny_dry_mass
Ba_shoot_dry_mass ~ ~ Ba_root_dry_mass
Ba_shoot_length ~ ~ Ba_root_length
Ba_root_dry_mass ~ ~ Ba_shoot_length
Ba_shoot_dry_mass ~ ~ Ba_shoot_length
NWG_shoot_dry_mass ~ ~ NWG_root_dry_mass
NWG_shoot_length ~ ~ NWG_root_length
NWG_root_dry_mass ~ ~ NWG_shoot_length
NWG_shoot_dry_mass ~ ~ NWG_root_length
NWG_shoot_dry_mass ~ ~ NWG_shoot_length
NWG_root_dry_mass ~ ~ NWG_root_length
Alf_shoot_dry_mass ~ ~ Alf_root_dry_mass
Alf_shoot_length ~ ~ Alf_root_length
Alf_root_dry_mass ~ ~ Alf_shoot_length
Alf_shoot_dry_mass ~ ~ Alf_root_length
Alf_shoot_dry_mass ~ ~ Alf_shoot_length
Alf_root_dry_mass ~ ~ Alf_root_length
'
model1.fit <- lavaan::cfa(model1, data=newdata, std.ov=TRUE)
summary(model1.fit, fit.measures=T, standardized=T, rsq=TRUE)
resid(model1.fit)
```

Model 2

```
model2 <- 'Agg.resp =~ EA_resp + FC_resp + Ba_resp + NWG_resp + Alf_resp
EA_resp =~ EA_progeny + EA_progeny_dry_mass + EA_progeny_wet_mass
FC_resp =~ FC_progeny + FC_survival
```

```
Ba_resp =~ Ba_root_length + Ba_shoot_length + Ba_shoot_dry_mass + Ba_root_dry_mass
NWG_resp =~ NWG_root_length + NWG_shoot_length + NWG_shoot_dry_mass +
NWG_root_dry_mass
Alf_resp =~ Alf_root_length + Alf_shoot_length + Alf_shoot_dry_mass + Alf_root_dry_mass
EA_progeny ~ ~ EA_progeny_dry_mass
EA_progeny_wet_mass ~ ~ EA_progeny_dry_mass
Ba_shoot_dry_mass ~ ~ Ba_root_dry_mass
Ba_shoot_dry_mass ~ ~ Ba_shoot_length
Ba_shoot_length ~ ~ Ba_root_length
NWG_shoot_dry_mass ~ ~ NWG_root_dry_mass
NWG_shoot_dry_mass ~ ~ NWG_shoot_length
NWG_shoot_length ~ ~ NWG_root_length
Alf_shoot_dry_mass ~ ~ Alf_root_dry_mass
Alf_shoot_dry_mass ~ ~ Alf_shoot_length
Alf_shoot_length ~ ~ Alf_root_length
'
model2.fit <- lavaan::sem(model2, data=newdata, std.ov=TRUE)
summary(model2.fit, fit.measures=T, standardized=T)
resid(model2.fit)
```

Model 3

```
model3 <- ' Agg.resp =~ EA_resp + Ba_mass + NWG_mass + Alf_resp
EA_resp =~ EA_progeny_wet_mass + EA_progeny_dry_mass
Ba_mass =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_mass =~ NWG_root_dry_mass + NWG_shoot_dry_mass
Alf_resp =~logAlfr + logAlfsh
'
model3.fit <- lavaan::sem(model3, data=newdata, std.ov=TRUE)
summary (model3.fit, fit.measures =T, standardized=T, rsq=TRUE)
resid(model3.fit)
```

Model 4

```
model4 <- ' Agg.resp =~ EA_resp + NWG_mass + Ba_mass
EA_resp =~ EA_progeny_wet_mass + EA_progeny_dry_mass
Ba_mass =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_mass =~ NWG_shoot_dry_mass + NWG_root_dry_mass
Agg.resp ~ logF2 + logF3
'
model4.fit <- lavaan::sem(model4, data=newdata, std.ov=TRUE)
summary(model4.fit, fit.measures=T, standardized=T)
resid(model4.fit)
```

Model 5

```
model5 <- ' Agg.resp =~ EA_resp + Ba_mass + NWG_mass
EA_resp =~ EA_progeny_wet_mass + EA_progeny_dry_mass
```

```
Ba_mass =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_mass =~ NWG_root_dry_mass + NWG_shoot_dry_mass
Agg.resp ~ logPHC
'
model5.fit <- lavaan::sem(model5, data=newdata, std.ov=TRUE)
summary (model5.fit, fit.measures =T, standardized=T)
resid(model5.fit)
```

Model 6

```
model6 <- 'Agg.resp =~ EA_resp + Ba_mass + NWG_mass + Alf_resp
EA_resp =~ EA_progeny_wet_mass + EA_progeny_dry_mass
Ba_mass =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_mass =~ NWG_root_dry_mass + NWG_shoot_dry_mass
Alf_resp =~ logAlfr + logAlfsh
Phys =~ Silt + Clay
Chem =~ Total_Nitrogen + Phosphorous + pH
Agg.resp ~ Phys + Chem
Total_Nitrogen ~ ~ Phosphorous
'
model6.fit <- lavaan::sem(model6, data=newdata, std.ov=TRUE)
summary(model6.fit, fit.measures=T, standardized=T, rsq=TRUE)
resid(model6.fit)
```

Model 7

```
model7 <- 'Agg.resp =~ EA_resp + Ba_resp + NWG_resp
EA_resp =~ EA_progeny_dry_mass + EA_progeny_wet_mass
Ba_resp =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_resp =~ NWG_root_dry_mass + NWG_shoot_dry_mass
Phys =~ Silt + logclay + WHC
Chem =~ Total_Nitrogen + Phosphorous + pH
Agg.resp ~ Phys + Chem
WHC ~ ~ logclay
Total_Nitrogen ~ ~ Phosphorous
'
model7.fit <- lavaan::sem(model7, data=newdata, std.ov=TRUE)
summary(model7.fit, fit.measures=T, standardized=T, rsq=TRUE)
resid(model7.fit)
```

Model 8

```
model8 <- ' Agg.resp =~ EA_resp + Ba_mass + NWG_mass
EA_resp =~ EA_progeny_wet_mass + EA_progeny_dry_mass
Ba_mass =~ Ba_root_dry_mass + Ba_shoot_dry_mass
NWG_mass =~ NWG_root_dry_mass + NWG_shoot_dry_mass
Agg.resp ~ logclay + pH
logclay ~ ~ pH
```

,

model8.fit <- lavaan::sem(model8, data=newdata, std.ov=TRUE)
summary (model8.fit, fit.measures =T, standardized=T, rsq=TRUE)
resid(model8.fit)

To model the relationship between each endpoint and PHC variable

logistic_model <- drm(newdata$EA_progeny [1:50] newdata$TotalPHC[1:50], fct =LL.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$EA_progeny_wet_mass[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$EA_progeny_dry_mass [1:50] newdata$TotalPHC[1:50], fct = LL.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$FC_survival[1:50] newdata$TotalPHC[1:50], fct = LL.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$FC_progeny[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$Ba_shoot_dry_mass[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)
logistic_model <- drm(newdata$Ba_root_dry_mass[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$Ba_shoot_length[1:50] newdata$TotalPHC[1:50], fct = LL.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$Ba_root_length [1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()), linreg = FALSE)

logistic_model <- drm(newdata$NWG_shoot_dry_mass[1:50] newdata$TotalPHC[1:50], fct

= W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$NWG_root_dry_mass [1:50] newdata$TotalPHC[1:50], fct
= W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$NWG_shoot_length[1:50] newdata$TotalPHC[1:50], fct =
W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$NWG_root_length[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$Alf_shoot_dry_mass[1:50] newdata$TotalPHC[1:50], fct =
W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$Alf_root_dry_mass[1:50] newdata$TotalPHC[1:50], fct = W1.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$Alf_shoot_length[1:50] newdata$TotalPHC[1:50], fct = EXD.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

logistic_model <- drm(newdata$Alf_root_length[1:50] newdata$TotalPHC[1:50], fct = LL.3())
mselect(logistic_model, list(LL.3(), LL.4(), LL.5(), W1.3(), W1.4(), W2.4(), baro5(),EXD.3()),
linreg = FALSE)

To obtain IC_{25} and IC_{50} values from model
select the best model for each endpoint and apply the "ED" function logistic_model_ED<-
ED(logistic_model, c(25, 50), interval = c("delta"))
plot(logistic_model,type="all")

To fit cumulative distribution of IC_{25} values
IC_{25} values were saved as study125#

IC25_lognorm1 <- fitdist(newdata$study125[1:8],"lnorm")
summary(IC25_lognorm1)

```
IC25_lognorm1 <- fitdist(newdata$study125[1:8],"gamma")
summary(IC25_lognorm1)

IC25_lognorm1 <- fitdist(newdata$study125[1:8],"exp")
summary(IC25_lognorm1)

cdfcomp(IC25_lognorm1,addlegend=FALSE,horizontals=FALSE,fitcol="black",
xlab="Total PHC", xlim=c(0,1400), lwd=3, cex=1.4, main="", cex.lab=1.2,
ylab="Cumulative Distribution")
abline(v=256.48,lty=3,lwd=2)
abline(v=283.75,lty=2,lwd=2)
```

Appendix C

Permission to use Image: Flow chart for the basic steps of SEM: (Rex B. Kline (2002))

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Best wishes, Angela

Appendix D

Properties of Oil Types (Information assessed from Klassen (NOAA, 2016))

Hello Idunnuoluwa,

Yes, we give you permission to use the table in the PDF document Oil Spills that you found on our website provided you reference our website. Best of luck with your thesis!

Sincerely, Nicole M. Hancock B.Ed. Distinction PGD Executive Director Safe Drinking Water Foundation Registered Canadian Charity #868384892RR0001 #1 - 912 Idylwyld Drive North Saskatoon, SK S7L 0Z6

Ph. 306-934-0389 Fax 306-934-5289 E-mail: info@safewater.org Website: www.safewater.org

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